Energetic Aspects of Brain Size Evolution:

Testing the Expensive Tissue Hypothesis in Mammals

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Summary

Human brains are unusually large among mammals. On the other hand, brain tissue is very expensive metabolically. The most widely accepted hypothesis on how our ancestors paid for the metabolic costs of brain size increase, the Expensive Tissue Hypothesis, proposes an energetic trade-off between brain and other tissues with high metabolic demands, especially the digestive tract. So far, the main evidence of comparative support for this hypothesis was a negative correlation between relative brain mass and relative digestive tract mass in anthropoid primates. However, due to limitations in the available morphological data, all previous-to-date published studies had to rely on combining various sources of brain size and organ mass data, which may have biased their results. Using a revised compilation based on the same original source of digestive tract data and more recently available sex-specific brain size data, we could not confirm a negative correlation between brain and gut mass in anthropoid primates.

The main aim of this study was to test the Expensive Tissue Hypothesis in both primates and mammals more generally, using reliable data. To do so, I collected morphological data of 454 mammalian specimens directly from dissections following a strict protocol. For analyses, animals preserved in alcohol, immature specimens, pregnant females and pathological individuals were excluded. The final sample contained 100 species, including 23 primate species.

Controlling for the effects of body size and phylogenetic non-independence, we found that brain size was not negatively correlated with the mass of the digestive tract or any other expensive organ, thus refuting the Expensive Tissue Hypothesis. However, we also found that the sizes of brains and adipose depots were negatively correlated in mammals, indicating that encephalization and fat storage may be compensatory strategies to buffer against starvation. Here, we argue that these two strategies can only be combined if fat storage does not unduly hamper

locomotor efficiency, as it happens in humans and aquatic mammals. We propose that human encephalization was facilitated by a combination of strategies to stabilize energy inputs and the redirection of energy from locomotion, growth and reproduction.

Zusammenfassung

Das Gehirn des Menschen ist im Vergleich mit anderen Säugetieren ungewöhnlich gross. Dies ist umso erstaunlicher, als dass Hirngewebe eine hohe Stoffwechselrate hat. Eine breit akzeptierte Hypothese, die erklärt, wie die metabolischen Kosten eines grösseren Gehirns im Verlauf der menschlichen Evolution bezahlt werden konnten, ist die sogenannte „Expensive Tissue Hypothese“. Sie postuliert einen energetischen Kompromiss zwischen Gehirn und anderen Organen mit hohen metabolischen Bedürfnissen, wie z.B. dem Verdauungstrakt. Diese Hypothese beruhte wesentlich auf einer negativen Korrelation zwischen Gehirngewicht und dem Gewicht des Verdauungstraktes bei Primaten. Die bisher publizierten morphologischen Daten erlaubten jedoch nicht, Gehirn- und Organgewichte derselben Individuen zu vergleichen, weshalb die bisherigen Ergebnisse mit Vorsicht zu interpretieren sind. In einer erneuten Analyse, die auf denselben Organmassen, aber neu publizierten Hirn- und Körpergewichten beruht, konnten wir eine negative Korrelation zwischen Gehirn- und Verdauungstrakt bei Primaten nicht bestätigen.

In der vorliegenden Arbeit sollte die Expensive Tissue Hypothese bei Säugetieren und Primaten überprüft werden. Dazu sezierte ich 454 Säugetierkadaver nach einem strikten Protokoll. Für die Analyse wurden in Alkohol aufbewahrte, schwangere und kranke Tiere, sowie Individuen mit unvollständigen Messreihen ausgeschlossen. Der endgültige Datensatz enthält 100 Arten, einschliesslich 23 Primatenarten. Die vergleichende Analyse korrigiert für unterschiedliche Körpergrössen und Verwandtschafts-Effekte. Die Resultate zeigen, dass das Gehirngewicht weder mit dem Verdauungstrakt noch mit anderen teuren Organen negativ korreliert, und zwar sowohl bei Säugetieren insgesamt als auch bei Primaten, Carnivoren oder Rodentia als Gruppen. Damit ist die Expensive Tissue Hypothese als allgemeingültiges Prinzip bei Säugetieren widerlegt.

Allerdings fand sich eine signifikante negative Korrelation zwischen Gehirngrösse und der Menge an gespeichertem Fett bei Säugetieren. Dieses Ergebnis lässt vermuten, dass kognitive Fähigkeiten und Fettspeicherung kompensatorische Strategien sind, um Perioden von Nahrungsknappheit zu überstehen. Diese zwei Strategien können nur dann kombiniert werden, wenn das gespeicherte Fett die Beweglichkeit und Effizienz der Fortbewegung nicht zu stark behindert, wie vermutlich beim Menschen und einigen wasserlebenden Säugetieren wie Walen oder Robbenartigen. In Bezug auf die Evolution des Menschen schlagen wir vor, dass die Vergrösserung des Gehirns im Pleistozän durch eine aussergewöhnliche Kombination von Faktoren ermöglicht wurde, die aber einzeln bei anderen Säugetiergruppen auch beobachtet werden können. Dabei spielten vermutlich insbesondere die Stabilisierung der Energiezufuhr auf einer höheren Ebene, die Versorgung der Mütter und Jungtiere durch Gruppenmitglieder, und die Effizienz der zweibeinigen Fortbewegung eine zentrale Rolle.

Curriculum vitae

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- German School of Barcelona, Spain (1999) – Abitur and Selectividad (Spanish university access exam)
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- University of Barcelona, Spain (2006) – MSc in Experimental Biology
    - Thesis: "Learning and feeding efficiency in urban pigeons (Columba livia): the cost of being a juvenile
- University of Barcelona, Spain (2007) – MSc in Animal Biodiversity
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- University of Zurich (2012) – PhD in Evolutionary Biology
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Lectures:

- 11th Conference of the Gesellschaft für Primatologie (Hannover, Germany 2009) – Oral presentation: "A revision of the Expensive Tissue Hypothesis"
- 78th annual meeting of American Association of Physical Anthropologists (Chicago, USA 2009) – Poster: "The Expensive Tissue Hypothesis revisited"
- 3rd meeting of the European Federation of Primatologists (Zurich, Switzerland 2009) – Oral presentation: "Testing the validity of the Expensive Tissue Hypothesis in mammals"
- 23rd Congress of the International Primatological Society (Kyoto, Japan 2010) – Oral

presentation: "The Expensive Tissue Hypothesis in primates – New results" (Presentation awarded with the Honorable Mention prize in the student competition)

- 12th Conference of the Gesellschaft für Primatologie (Utrecht, The Netherlands 2011) – Oral presentation: "The Expensive Tissue Hypothesis in primates – New results"
- 4rd meeting of the European Federation of Primatologists (Almada, Portugal 2011) – Oral presentation: "The Expensive Tissue Hypothesis in primates and in mammals"
- 81st American Association of Physical Anthropologists (Portland, Oregon, USA 2012) – Oral presentation: "Evolution of human encephalization: evidence from a comparative analysis of brain size and fat storage"

Publications:

- Ana Navarrete, Carel van Schaik and Karin Isler (2011) "Energetics and the evolution of human brain size" Nature, DOI: 10.1038/nature10629

Occupational activities:

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- Since February 2011: temparilly employed in the collection of the Anthropological Institute and Museum under the supervision of Dr. Christopher Zollikofer.

Grants during doctorate studies:

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Chapter 1: Introduction

1.1. Brain size evolution

Brain tissue is energetically expensive and requires more energy per unit weight than many other somatic tissues (Mink *et al*. 1981, see also Table 1.1). The proportion of metabolic energy shunted to the brain in resting state is around 13% and 20% in adult chimpanzees and humans, respectively, for an organ that only constitutes 1-2% of the total body mass (Mink *et al*. 1981; Holliday 1986). The brain energetic consumption rate also tends to be higher for immature individuals, whose developing brains make up a higher proportion of total body weight. Human neonates, for example, devote well over 60% of their metabolism to their brains (Holliday 1986, see Figure 1.1). This energetic expense is additionally aggravated by the fact that, in contrast to that of other organs, the brain's energy consumption cannot be temporarily reduced (Karasov *et al*. 2004; Bauchinger *et al*. 2005). Therefore, we would expect that the high proportion of energy necessarily allocated to develop and maintain brain size must impose serious constraints on the selective process of increasing brain mass, i.e. encephalization.

Generally, we focus on the study of the evolution of overall brain size rather than on the evolution of specific regions. This is partly because brain composition across species of a given lineage shows obvious regularities that suggest that changes in one region are necessarily linked to a fairly large extent to changes in others (Finlay and Darlington 1995; Jerison 2001). Using overall brain size is also warranted from the energetic perspective, because an individual's cognitive performance is linked to the size of its whole brain, not only between species (Jerison 1973; Deaner *et al*. 2007), but also within species, as observed in humans (McDaniel 2005).

Table 1.1: Percentage of total body mass, total metabolic rate and metabolic consumption assigned to each tissue in humans. These measurements are based on an average individual of 60.5 kg of body mass with a total metabolic consumption of 12'457 mL O_2/h (Rolfe and Brown 1997).

	% Body mass	% Metabolic rate	Organ metabolic consumption (mL O_2/g*h)
Liver	2.0	17	1.75
Digestive tract	2.0	10	1.03
Kidney	0.5	6	2.47
Lung	0.9	4	0.92
Heart	0.4	11	5.66
Brain	2.0	20	2.06
Skeletal muscle	42.0	20	0.10

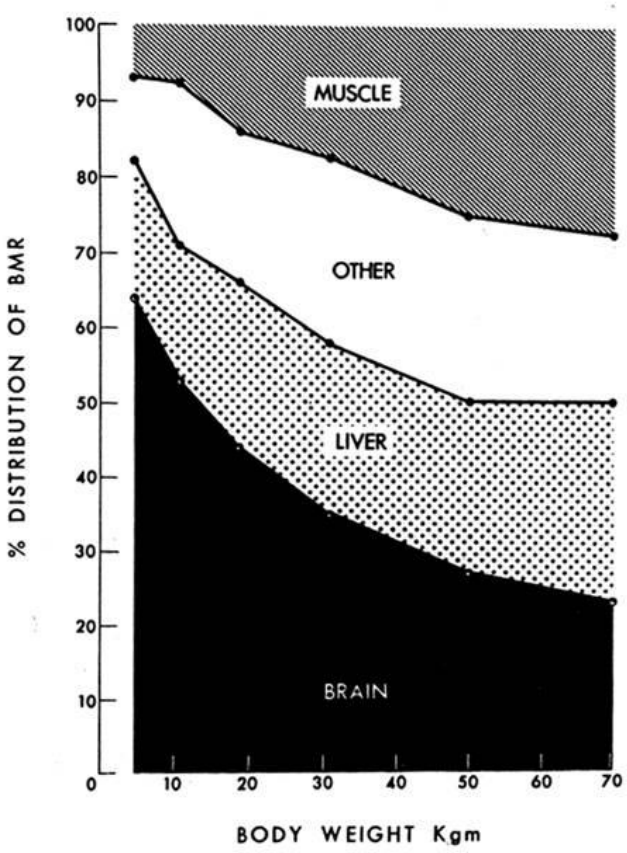


Figure 1.1: Organ metabolic consumption as percentage of total basal metabolic rate (BMR) in humans (Holliday 1986).

Between mammal and bird species, a large variation in the relative size of the brain can be observed (where "relative" means after statistically removing the correlation with body size, e.g. by the use of residuals). Explaining interspecific variation in relative brain size in these groups is considered important, as it corresponds to differences in intellectual or cognitive performance between species (Iwaniuk *et al*. 2001; Lefebvre *et al*. 2004; Sol *et al*. 2005; Deaner *et al*. 2007). Looking at brain size variation, we can also have a better insight into how encephalization costs may have been balanced and overcome by cognitive benefits during evolution in high encephalized species. This is a question of great interest in anthropology: human brain size is roughly three times that of our closest relatives, the common and pygmy chimpanzees, and of early hominins, the australopithecines. This spectacular increase, which happened in a rather short period of time, is one of the core research trends in human evolution.

However, over the last decades, the numerous studies in birds and mammals have mostly attempted to explain the interspecific variation in brain size focusing on the beneficial aspects of encephalization and its associated enhanced cognitive abilities only. Species would have improved foraging efficiency, especially by extracting invisible food from a hard matrix (Parker 1990; Byrne 1997), would have been able to remember the location of spatio-temporally varying, ephemeral food items, and to navigate in the landscape to exploit the patches as efficiently as possible (Milton 1981), and, most prominently, would have been able to deal with the challenges of competition and cooperation imposed by living in social groups (Byrne and Whiten 1988; Barton and Dunbar 1997; Dunbar 1998; Tomasello 2000; de Waal 2003; Dunbar 2003; Barrett and Henzi 2005). The latter idea in particular is often invoked to account for the remarkable

increase in brain size during human evolution (Leonard *et al*. 2007). The cost side of brain evolution has, in comparison, received much less attention.

The present project was built on the thesis that a more complete explanation of the taxonomic variation in relative brain size, and thus of cognitive evolution, will be achieved if we incorporate both costs and benefits in the equation. Until recently, the cost side of brain evolution had not been integrated into mainstream theory on cognitive evolution (but see Martin 1981; Aiello and Wheeler 1995; Martin 1996), even when the consideration of the costs associated to encephalization would complement ideas positing benefits to larger brains, revealing the conditions under which such positive selection pressures would be able to produce actual increases in brain size. Additionally, we predicted that interspecific variation in the ability to sustain these costs should explain much better interspecific variation in brain size than the variation in benefits, because brain size enlargement can only be afforded in circumstances where the costs are somehow compensated through metabolic, physiological and/or behavioral pathways. Summing up, we would only expect an increase in encephalization in situations where the extra requirements of developing and maintaining enlarged brains can be compensated by the benefits associated to enhanced cognition (Aiello and Wheeler 1995; Isler and van Schaik 2006).

In logistical terms, selection experiments involving a broad range of species are not practical, especially in species with long life spans such as primates. Short-lived species, such as mice, on the other hand, may show different reaction norms and would therefore not be conclusive for understanding human brain size evolution. Therefore, hypotheses concerning brain evolution must be tested using comparative analyses.

1.2. The Expensive Brain framework

Research on the cost-side of encephalization has been scarce until recently. Nevertheless, a few years ago, Isler and van Schaik (2009) combined all previously published hypotheses on the cost-side of brain evolution into the Expensive Brain framework (see Figure 1.2).

According to this framework, there are two non-mutually exclusive pathways which would allow an increase in brain size. Larger brains could evolve, first, under circumstances which allow an increase in the energy budget, generating additional energy which can be channeled into brain growth and maintenance, and, second, by changing the allocation of the energy which is already generated by the system, reducing other functional costs and the size of other tissues. This basic framework makes several strong predictions, in reference to both development and adult performance, which will be explained in the following sections.

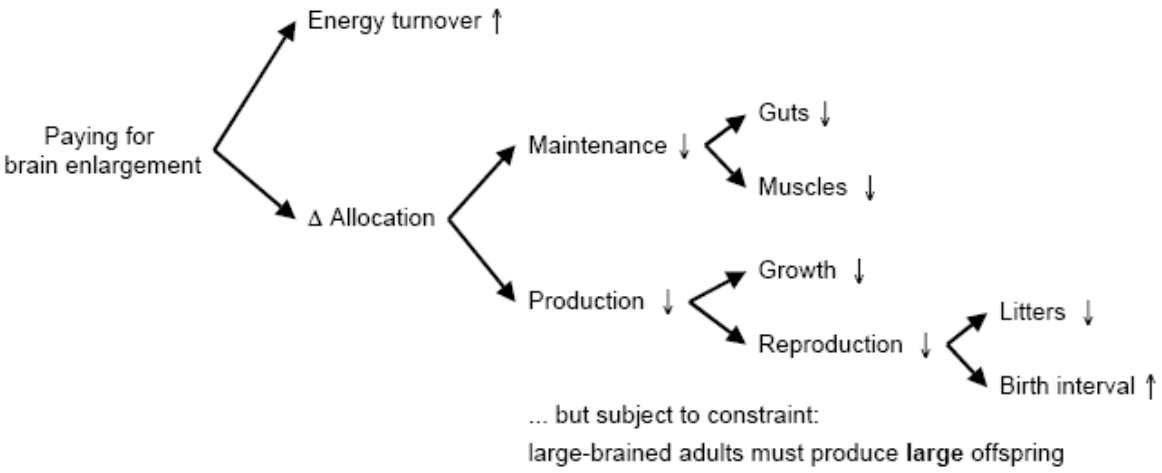


Figure 1.2. Expensive Brain Framework (Isler and van Schaik 2009).

1.2.1. Increasing metabolic rate

One way to facilitate an increase in brain size is to increase the amount of energy in the system and invest the energy surplus in more brain tissue (see Figure 1.2). Hypotheses about this first pathway were first presented in the earlier 80s, when the so called Metabolic Constraint Hypothesis was put forward by two authors (Armstrong 1983; Hofman 1983). This hypothesis suggested that brain size enlargement should be possible with an increase of the daily energy budget, i.e. the amount of energy entering the system (by increasing ingestion, accelerating metabolism, etc). This hypothesis predicted, therefore, a positive correlation between brain size and metabolism, measured as basal metabolic rate (BMR), after controlling for body size effects.

It was not until recently that new studies could corroborate its validity in mammals (see Figure 1.3), but not in birds (Isler and van Schaik 2006). On the other hand, the extent by which an increase in metabolism may have contributed to the enlarged human brain has been debated, as the resting energy demands of humans are similar to those of other mammals of the same size (Leonard *et al*. 2007).

The major problem of testing the Metabolic Constraint Hypothesis lies in the difficulty of measuring the daily energy budget in vertebrates. The BMR is only a proxy measure of metabolism, which is defined as the post-absorptive metabolic rate of non-reproductive adults during rest and in a thermo-neutral state. The use of BMR as a measure of daily energy budget has been intensively debated, as it is a measure taken under very restricting conditions and does not indicate the extent of energy that is being used by an individual under natural, active conditions. Unfortunately, measures with a better approach to real metabolic expenditure, such as

the resting metabolic rate (RMR) or the field metabolic rate (FMR), are less frequently reported in the literature (Nilsson 1996).

In lack of the availability of a better measure, we used BMR values as a proxy of metabolism to analyze its role on brain evolution in mammals, even though published compilations of BMR measurements are not fully reliable in some species because of inaccuracies of methodology (Genoud, in prep.).

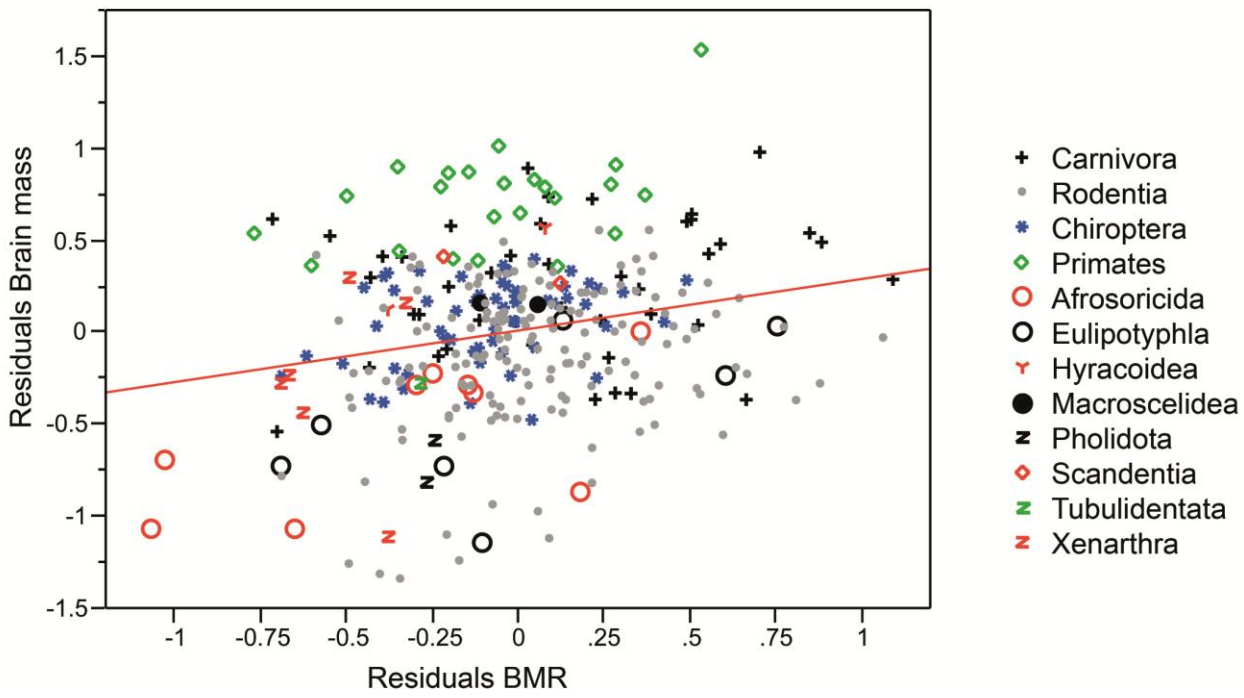


Figure 1.3: Correlation between brain size and basal metabolic rate (BMR), after correcting for body size effects, in mammals (raw data: N=313, R^2=0.053, P<0.0001, independent contrasts: N=312, R^2=0.026, P=0.005, Isler and van Schaik 2006).

1.2.2. Changing energy allocation

The second pathway to compensate the increasing costs of encephalization, as announced by the Energy Trade-off Hypothesis, would consist on relocating some energy to the brain, after reducing the costs of maintenance of other tissues, and/or by reducing the costs of reproduction and development (see Figure 1.2). This can be achieved through balancing the competing brain demand for energy with the energetic demand of other major somatic functions, i.e. maintenance of other tissues, growth rate of immature individuals or reproductive rate in adults.

One aspect of this pathway, namely the reduction of the costs of maintenance of other tissues to increase brain mass, is included in the Expensive Tissue Hypothesis (Aiello and Wheeler 1995), the most widely known contribution to the cost-side perspective of brain evolution.

1.2.3. The Expensive Tissue Hypothesis

In 1995, Aiello and Wheeler presented the Expensive Tissue Hypothesis, which proposes that, during human evolution, changes in brain size were compensated by concomitant changes in the size of the digestive tract (Aiello and Wheeler 1995).

In addition to the brain, high energy consumption rates have been found for some other organs (e.g. the digestive tract, liver, kidney, heart) and to some extent muscle tissue (see Table 1.1). Although muscles are much less expensive to maintain than brain tissue, they use a significant part of the resting metabolism (20% in humans, 30% in rats; Rolfe and Brown 1997). The digestive organs were favored by these authors as potential candidates for a trade-off with brain tissue because a reduction of digestive tissue could be enabled by improved digestibility of

their diet, whereas the functionality of other expensive organs would be unsustainably compromised with shrinking size. Muscle mass, on the other hand, would have to suffer an enormous reduction to liberate enough energy to increase brain size (Aiello and Wheeler 1995). In their publication, Aiello and Wheeler provided support for their hypothesis using a sample of 18 anthropoid species, including *Homo sapiens*, where a negative correlation between brain size and digestive tract mass could be observed (see Figure 1.4, Aiello and Wheeler 1995). This correlation persisted even when the last species was excluded from the sample (Aiello *et al.* 2001).

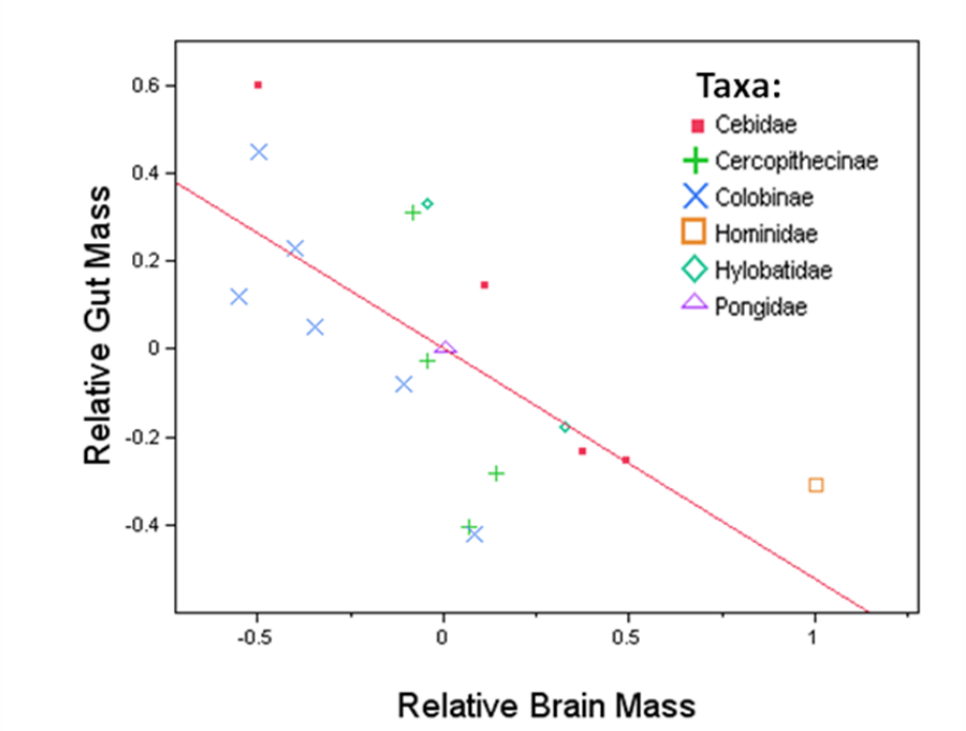


Figure 1.4: Correlation between relative brain mass and relative gut mass in anthropoids (Aiello and Wheeler 1995, N=18, R^2=0.476, P=0.0015)

The logic underlying the Expensive Tissue Hypothesis and its elegance contributed to the broad acceptance of the hypothesis among the academic community in different fields (Santoro *et al*. 2006; Leonard *et al*. 2007; McGill 2008; Mau *et al*. 2009; Babbitt *et al*. 2011; Burns *et al*. 2011; Pfefferle *et al*. 2011) and, since its publication, it has played a major role in the understanding of human brain evolution (Leonard *et al*. 2007). As such, a trade-off should apply in various lineages. Several authors have tried in the last years to test the Expensive Tissue Hypothesis in primates and other groups, but, so far, the results have been ambiguous.

The most promising results supporting the Expensive Tissue Hypothesis have been found in primates. Joining Aiello and Wheeler's analyses on anthropoid primates, a recent study in strepsirrhines showed that relative brain mass and relative intestine mass correlated negatively in 9 species of lemurs and lorises (Barrickman and Lin 2010). Also, using the Expensive Tissue Hypothesis as a reference, Fish and Lockwood (2003) tested the correlation between brain size and diet quality in primates, as diet quality is known to be inversely related to gut dimensions, and their results indicated that diet quality correlated positively with brain size. However, diet quality is also positively correlated with BMR, and, therefore, the found correlation could also be attributed to direct metabolic constraints. More recently, Allen and Kay (2011) challenged the validity of the Expensive Tissue Hypothesis when they did not find a correlation between dietary quality and brain size in platyrrhine primates, which are characterized by a high variability in relative brain size.

In fish, Kaufman *et al.* (2003) found a negative correlation between brain mass and digestive tract mass. This study is often cited as support for the universality of the Expensive Tissue Hypothesis, even when it was based on a reduced sample of 3 fish species that are not

closely related to each other. In bats, the Expensive Tissue Hypothesis was not validated: frugivores showed relatively larger brains than insectivores, even when an insectivore diet is normally considered of a higher quality (Jones and MacLarnon 2004). Another study on bats presented a trade-off between brain mass and reproductive tissues (Pitnick *et al*. 2006), which was later dismissed as spurious (Lemaitre *et al*. 2009). Finally, Isler and van Schaik (2006) found a negative correlation between brain mass and pectoral muscle mass in birds, linking brain evolution with costs associated to locomotion.

However, all these studies, including Aiello and Wheeler (1995), suffer from similar methodological bias affecting the morphological data used in the analyses. At the time of their publication, morphological data on organ mass and on brain size from the same individuals were not commonly available, and samples of species with complete measurements were very limited (Pitts and Bullard 1968; Schoenemann 2004). Therefore, most of these studies relied on mixed data on digestive tract, brain and body mass extracted from different sources, and thus different individuals. This combination of data from different sources may have produced increased error variation, particularly in species where the matched variables were taken from individuals of different sex. These methodological problems have already been the focus of criticism and, as it was recognized by the authors of the Expensive Tissue Hypothesis in a later publication (Aiello *et al*. 2001), warranted the need for further reevaluation of the found correlations.

The aim of this project was to overcome these methodological problems. First, we wanted to obtain new morphological measures of a large number of mammal species directly from dissections, following a standardized protocol which could be applied in future studies, and use these measures to test the Expensive Tissue Hypothesis in primates and mammals. Second, we

aimed to extend our examination to other possible trade-offs amongst other expensive organs, as predicted by the more general Energy Trade-off Hypothesis (Isler and van Schaik 2006).

1.3. Methodological considerations

Obtaining reliable measures of organ mass does not only depend on a standardized protocol. Specimens, especially primates, are difficult to access, and harvesting wild-living animals for morphological studies is not an option due of ethical concerns, particularly in the case of endangered species. Therefore, for our morphological studies, we mainly had to rely on museum collections. These collections usually have a good representation of local fauna and, thanks to collaborations with zoological gardens, may include some interesting and valuable exotic species. Museums whose interest is focused on osteological and skin collections are also willing to allow researchers to collect morphological data on soft tissues using invasive techniques. However, some characteristics of the specimens need to be accounted for. Pooling wild and captive specimens may result in a biased sample composition. Moreover, details of preservation treatments may also affect measurements, and must therefore be considered.

1.3.1. Effects of preservation methodology

Ideally, both body mass and the mass of the internal organs of an individual should be determined shortly after death, before tissues start to degenerate. However, various practical reasons may prevent access to or investigation of recently deceased specimens, especially animals that are not kept in laboratories. For animals that cannot be processed immediately, various preservation methods are applied to prevent tissue degeneration for short or extended periods of time and to maintain the characters of interest until their study (e.g. Stoddart 1989;

Simmons 1995). However, these treatments are known to cause changes in organ mass (e.g. Kruska and Steffen 2009). The relevance of these changes depend on the type and the duration of treatment (Florian 1990) and should be taken into consideration when stored specimens are used in morphological studies (Shields and Carlson 1996).

In museums, the two most common preservation methods for whole specimens are storage in the cold, and immersion in alcohol or formalin solutions. The first option, storage at -4 to -20 °C, is recommended for short-term storage (hours to years) of animals which will undergo later taxidermical or osteological preparation, or be used in gross anatomical studies. Cold storage is, however, not adequate for samples of histological analyses, because freezing causes histological damage due to the formation of intracellular ice crystals which pierce cell membranes and organelles (Florian 1990). On the other hand, cold storage does not stop tissue deterioration completely, although it slows down bacterial and fungical growth (Florian 1990), and may often cause desiccation if specimens are not sufficiently isolated and/or are stored over long time periods (pers. obs., Florian 1990).

The second treatment, wet storage in alcohol or formalin solutions, is recommended for long-term preservation (up to decades) and allows detailed anatomical or histological studies for even after years of treatment (Florian 1990). For specimens of small to medium body size, or for individual organs, immersion in 70-90% alcohol solution is usually preferred over formalin fixation, because the latter requires constant supervision and special conditions of storage to avoid low pH values that can eventually harden soft tissues and soften bones (Sturgess and Nicola 1975). The success of alcohol preservation depends on quick diffusion of the alcohol to all tissues of the body. Thus, a common practice to facilitate diffusion of the preservative is the

removal of the viscera prior to storage (Rabinowitz 2000), rendering those specimens useless for inner anatomy studies. Alternatively, the abdominal cavity can be opened to accelerate absorption, or alcohol can be injected into the internal body cavities before immersion. Wet specimens (both in alcohol and in formalin) are known to lose weight during long-termed preservation periods, because both the preservatives dehydrate tissue (Glenn and Mathias 1987).

Documentation concerning wet preservation techniques has been minimal for most collections of specimens, and not until the last two decades have museums and other institutions begun to make an effort to apply a general methodology for the maintenance of their collections (Cato 1990). Although alcohol preservation is one of the most common preservation methods, other chemical compounds such as formalin are also frequently used, using sometimes mixed preservation methods which are difficult to replicate and control. Studies in macroinvertebrates and fish, which compared differences in body mass between specimens preserved in alcohol, and specimens first fixated in formalin and then preserved in alcohol, yielded mixed results, with some showing that the effects on body mass depended on the timing of the different treatments (Fox 1996; Peterson and Vanderkooy 1996; Shields and Carlson 1996; Wetzel *et al*. 2005; Nadeau *et al*. 2009), and other showing that body mass loss seemed to be similar to those specimens that were preserved in alcohol from the start (Wetzel *et al*. 2005). Whether or not fixation with formalin was applied to a specimen is easily detected as a distinctive formalin odor persists, even after long preservation periods (pers. obs.).

Effects of both preservation treatments, cold and wet, on collected specimens have been studied predominantly in fish (e.g. Fox 1996; Peterson and Vanderkooy 1996; Shields and Carlson 1996; Nadeau *et al*. 2009), but also in other vertebrates (Lee 1982; amphibians: Paulov

1988; Deichmann *et al*. 2009; lizards: Vervust *et al*. 2009) and invertebrates (Howmiller 1972; Wiederholm and Eriksson 1977; Leuven *et al*. 1985; Distefano *et al*. 1994; Reese *et al*. 1996; Wetzel *et al*. 2005). Studies on the effect of preservation treatments on birds and mammals, however, have been scarce (Schultz 1919; Downing 1945). In all groups, studies focused mainly on external measurements such as body and limb length and skin or feather coloration, but less on changes in body mass. Changes in the mass of inner organs are almost inexistent (with the exception of a study on the impact of preservation on gut content; Hay 1981; Kruska and Steffen 2009). Therefore, we considered that an evaluation of the body and organ mass changes caused by preservation treatment in mammals was warranted and of interest for this study.

1.3.2. Effects of sex, provenience and habitat on the correlation between brain size and the masses of other tissues

Organ morphology has been shown to be influenced by factors such as sex, living conditions, diet and habitat (Foley and Cork 1992; Hammond *et al*. 2001; O'Regan and Kitchener 2005), and it is therefore important to consider these factors while testing our hypotheses, to reduce error variation.

Regarding sex, we would expect physiological trade-offs to be more pronounced in females than in males, because the former are more affected by energy constraints due to offspring production (Isler and van Schaik 2009). As captivity effects are rather unpredictable (most animals gain weight in captivity, but only under good husbandry conditions; O'Regan and Kitchener 2005), we would also expect possible trade-offs to be more pronounced in wild-caught

specimens, although it may be argued that these specimens are also more affected by seasonality effects on fat storage, which would influence body size (e.g. Altmann *et al*. 1993).

These factors have not been taken into consideration previously in the literature because of limitations of the published samples, or the limited variability of mammalian carcasses. In the records, sex is usually reported, but including specimens of only one sex would have resulted in a drastic reduction of the sample size. Provenience, i.e. details of the living conditions of the specimens, is rarely reported and analyses comparing physiological trade-offs in wild and captive individuals have not been yet conducted. Our aim here was to collect this information and use it to test the robustness of our results by splitting samples into subsets according to sex, provenience and habitat.

1.4. Aims and overview

This thesis is part of a larger project which aimed to test various predictions flowing from the energy-cost perspective on brain size evolution in different taxonomic groups. Within this project, I focused on testing the Expensive Tissue Hypothesis and its more general version, the Energy Trade-off Hypothesis, in eutherian mammals.

Specifically, my aims were:

1. To re-evaluate the Expensive Tissue Hypothesis in primates and mammals using published datasets and phylogenetic comparative methods, in order to assess the sample size needed to achieve sufficient power of the tests. This is reported in Chapter 2, and in the section on power analyses in Chapter 4.1.

2. To experimentally assess changes in organ mass caused by different preservation methods in a sample of laboratory mice. Depending on the outcome of this experiment, it would be feasible to control for the effects of preservation treatment in the other preserved specimens, and maximize the comparative sample. These experiments are reported in Chapter 3.
3. To collect a new reliable dataset of organ mass data in mammals directly from dissections. A single dissector should measure both expensive organs and other tissues, such as muscle and fat, using a standardized protocol. The number of individuals for each species was to be maximized according to the availability of specimens, up to a number of about 10 per species. Special emphasis was put on the collection of primate specimens. The methodology that I used to perform these dissections is described in Chapter 4.2.
4. To test the Expensive Tissue Hypothesis in mammals with the new dataset, using state-of-the-art statistical methods to control for phylogenetic inertia. If possible, these tests would take sex, provenience and habitat into consideration. Methods and results are reported in Chapters 4 and 5, respectively.
5. To test the correlation between daily energy budget (proxied by the basal metabolic rate, BMR), brain mass and the mass of other tissues, and the influence of metabolism on the correlation between brain and other tissues. These results are reported in Chapter 5.6.
6. To integrate these results into the Expensive Brain Framework for a synthesis of the cost-side perspective. This is done in the general discussion in Chapter 6.

Chapter 2: Evaluation of published datasets

2.1. Datasets of mammals

Ideally, testing the Expensive Tissue Hypothesis should rely on data collected with the same methodological procedures and with all organ measurements taken from the same individuals. However, previous to this project, matching visceral organ mass data had only been collected for a small number species, and the combination of different sources, using different dissection protocols, was the only option to obtain datasets large enough for these tests.

Published datasets including measurements of both brain mass and digestive tract mass (with or without measurements of other organs) are rare in the literature. Within mammals, the two most elaborate datasets are those of Crile and Quiring (1940) and Pitts and Bullard (1968).

Crile and Quiring's (1940) dataset includes incomplete organ measures from 3690 specimens of a large number of vertebrate and invertebrate species from all over the world. The main achievement of this dataset was that it incorporated relevant data about the age, the sex and the origin of most specimens. Age information permits the exclusion of juveniles, which have relatively larger brains and smaller guts than the adults. Origin information is also relevant because captive and wild-derived individuals may differ in organ masses (O'Regan and Kitchener 2005). However, although brain data is available for most specimens of this sample, digestive tract mass is only implemented for a subset of adult specimens, and complete measures of adult individuals are only available for 35 mammal species from all ranges of body size. The most serious drawback of this sample is the lack of a detailed description of the methodology regarding the dissection of the digestive tract. No detailed protocol is given and, very likely, the gut was not

(or not completely) emptied before weighing. Therefore, combining gut mass data from Crile and Quiring with data from other studies could be considered problematic.

The dataset of Pitts and Bullard (1968), on the other hand, only includes measures of 39 mammal species and is by far the most complete morphological dataset published, with measures of most relevant organs (with the exception of kidneys) for all species. It also provides measurements of whole and fat-free body mass. However, all species included in this dataset are small bodied, and some orders are poorly represented (e.g. only one species of primate). Additionally, the dissection protocol used for the collection of data does not allow combining it with data from other publications. Instead of reporting brain mass, the authors give central nervous system (CNS) mass, which includes brain mass and spinal cord mass. Although the spinal cord only accounts for a relatively small amount of total CNS mass, its inclusion makes the measure in this study incompatible with other sources. However, due to its consistent methodology, this sample was very useful to assess the statistical power of our planned analyses (see Chapter 4.1).

Apart from these two major datasets, other publications with detailed organ mass data from one or a few species are available in the literature. These studies usually do not give individual measures, but morphological averages for comparisons between populations, sexes, age classes and dietary treatments, using a sometimes unknown sample size. This makes it difficult to determine how the specimens' values were pooled into an average. These averages mask the existent variability between conditions or populations (Hammond *et al*. 2001), although they can still be used with some caution in comparative analyses, if the dissection protocol is

poorly described. As it happened in the Crile and Quiring sample, data on the mass of fat deposits or fat-free body mass are usually not given.

In conclusion, evaluating the Expensive Tissue Hypothesis in mammals was not feasible with previously published data. The samples of specimens with both measures of brain size and organ mass were either very limited or incompatible between sources. Analyses within specific groups, such as primates, could therefore only be done so far using datasets which combined morphological measures from different sources (Aiello and Wheeler 1995; Isler and van Schaik 2006). Additionally, even when most authors were conscious about the fact that the combination of organ measures from different individuals, especially in cases of species with strong sexual dimorphism, may have biased their results, they could not have fully assessed if these bias could have been worsened by the inclusion of undetected juveniles in the averages.

2.2. Primate dataset

As commented before, due to the small number of primate species with both brain size and organ mass data from the same specimens available in the literature, studies on primate morphology have relied on combining different sources. The original dataset of anthropoid primates of Aiello and Wheeler (1995), which was used to postulate the Expensive Tissue Hypothesis, was also built as a combination of a dataset for brain size (Clutton-Brock and Harvey 1980) and a dataset for gut mass (Chivers and Hladik 1980, and unpublished annotations).

The source of relative brain size of the species used in Aiello and Wheeler (1995) was the Clutton-Brock and Harvey's (1980) dataset. Until the recent publication of a more complete dataset (Isler *et al*. 2008), this was the best available compilation of primate brain size data in the

literature. Here, body mass averages were given for females and males separately, but only one average of brain mass was given for each species. Per species, Aiello used the body mass average of both sexes to correct body size effects on brain size. Only for *Homo sapiens* were brain and body mass values taken from another resource (Aschoff *et al*. 1971), presumably because of the low body mass values in the Clutton-Brock and Harvey's dataset for this species (45 kg for males).

The Chivers and Hladik's (1980) dataset includes individual mass and surface area measurements from several components of the digestive tract (stomach, ileum, caecum and colon), as well as matching body mass in a sample of 157 mammal specimens, including the gut mass of 37 primate species. This source contains a layout error, which Aiello and Wheeler were able to correct for, as Chivers made the full list of measurements, complemented with unpublished data, available to Robert D. Martin and Leslie Aiello. Aiello and Wheeler found the negative correlation between relative brain mass and relative gut mass using only 18 anthropoid species from this dataset, though their calculated averages for each species were not published until several years later (Aiello *et al.* 2001, see Table 2.1). A reconstruction of Aiello and Wheeler's dataset, however, shows that the digestive tract averages used by these authors must have included juveniles or emaciated individuals in some species, whereas for other species it is unclear which individuals were used for the averages. Therefore, we found it necessary to first re-evaluate the Expensive Tissue Hypothesis in anthropoid primates using sources published in the literature.

2.3. Evaluation of the Expensive Tissue Hypothesis using bibliographical data

The original analysis of Aiello and Wheeler yielded a negative correlation between brain size and digestive tract mass (the sum of stomach, ileum, caecum and colon mass), controlling for the effect of body mass (N= 8, r=-0.69, P<0.001; Aiello and Wheeler 1995). This negative correlation persisted when *Homo sapiens* was excluded from the sample (N=17, r=-0.62, P=0.007, Aiello *et al*. 2001). Prior to the collection of new data, we conducted a detailed evaluation of the original study that supported the Expensive Tissue Hypothesis in anthropoid primates to test the consistency of these results.

2.3.1. The compiled dataset

In this evaluation of the Expensive Tissue Hypothesis, we used the Chivers and Hladik's (1980) dataset, making full use of current phylogenetic methodology and more accurate sex-specific brain volume and body mass data which has become available in recent years (Isler *et al*. 2008; van Woerden *et al*. 2010) and additional measures provided by J. van Woerden (pers. comm.).

Individual digestive tract measures were taken from Chivers and Hladik, after the exclusion of suspected emaciated and immature individuals. The original study correlated brain mass with gut mass, i.e. digestive tract mass, described as the sum of the masses of stomach, ileum, caecum and colon. In our re-evaluation, we tested the correlation between brain mass with 1) stomach mass, 2) intestine mass (described as the sum of ileum, caecum and colon) and 3) digestive tract mass.

Because brains and digestive tract measures were obtained from different specimens, a major challenge was to deal with the variability in body mass within species and the error of estimating the relative size of organs this produces. In order to obtain an optimum correspondence between digestive tract and brain specimens, we included only adults that were not emaciated, as compared to average body mass values for the respective sex (body mass less than 75% of the average mass). To preclude confounding effects of body mass dimorphism between the sexes, we adhered to the following rationale. First, we calculated the averages for the digestive tract variables for each species, recording whether the average included only females, only males or both sexes. Second, brain mass was paired to the digestive tract values as follows: for species with no sexual dimorphism in body mass (less than 10% difference between males and females), we took the average brain mass for both sexes; otherwise, we took the average brain mass for the same sex or the whole species, depending on the sex of the individuals included in the digestive tract averages. Our final sample included 23 anthropoid and 2 strepsirrhine species (see Table 2.1).

All variables were log-transformed. Phylogenetic regressions (PGLS) were run using pglm.estlambda in the CAIC package (Orme *et al*. 2009) in R (Team 2010) with the consensus tree from the 10kTrees project (Arnold *et al*. 2010). As the small sample size yielded unstable estimates of lambda in some cases (lambda not significantly different from either 0 or 1), we additionally ran all analyses with lambda set to 0 (raw data) or 1 (classic independent contrasts). The phylogenetic least-squares regression model included both body masses and the digestive tract mass as independent variables and brain mass as the dependent variable.

Table 2.1: The two datasets based on the digestive tract mass data from Chivers and Hladik.

		A: Dataset used by Aiello and Wheeler				**B: Revised dataset**												
		Digestive tract data		Brain data		Digestive tract data					Brain data							
											Female		Male			Adjusted sex		
Group	Species	BM	DT	BM	Brain	N_{ind}	BM	St	Int	DT	BM	Brain	BM	Brain	Dim	Sex	BM	Brain
Str	*Galago alleni*					1f	250	3	11	14	269	5	277	6	1.03	m/f	273	6
Str	*Galagoides demidoff*					1m	60	1	1	2	75	3	76	3	1.02	m/f	76	3
Pla	*Saguinus mystax*					1m	560	2	21	23	584	10	629	10	1.08	m/f	607	10
Pla	*Alouatta belzebul*					3f/1m	5050	94	274	368	5520	51	5525	55	1.00	m/f	5523	53
Pla	*A. seniculus*	4450	360	7250	58	1m	6150	116	325	441	5210	55	6668	55	1.28	m	6668	55
Pla	*Callicebus moloch*					2m	1165	7	32	39	887	17	935	18	1.05	m/f	911	17
Pla	*Cebus apella*	2890	110	2480	71	1f	2000	16	86	102	2489	64	3383	69	1.36	f	2489	64
Pla	*Lagothrix lagotricha*	8050	362	6300	96	1f/1m	8050	93	269	362	7020	89	7280	89	1.04	m/f	7150	89
Pla	*Saimiri sciureus*	740	39	665	24	1f/1m	990	6	39	45	743	24	860	24	1.16	m/f	802	24
Cat	*Cercopithecus cephus*	3338	120	3500	64	1f/2m	3567	26	102	127	2880	61	4290	66	1.49	m/f	3585	63
Cat	*C. neglectus*	4081	254	5480	71	1f/1m	7595	61	212	273	4130	61	8048	67	1.95	m/f	6089	64
Cat	*C. nictitans*					1m	6500	61	157	218	4260	67	6670	73	1.57	m	6670	73
Cat	*Erythrocebus patas*	11650	280	7800	107	1m	11650	54	226	280	6500	89	12400	97	1.91	m	12400	97
Cat	*Macaca fascicularis*	3175	149	5000	69	1f/2m	4400	38	167	205	3518	61	5360	65	1.52	m/f	4439	63
Cat	*Mandrillus sphinx*					1f	12300	123	641	764	12800	136	45000	159	3.52	f	12800	136
Cat	*Colobus polykomos*	7662	380	9400	77	2f	8465	190	173	363	6709	71	10600	78	1.58	f	6709	71
Cat	*Nasalis larvatus*	15880	598	15100	94	1m	15880	357	241	598	9730	85	19392	99	1.99	m	19392	99
Cat	*Presbytis melalophos*	6781	254	6650	80	3f/3m	6537	122	134	256	6567	61	6554	72	1.00	m/f	6561	66
Cat	*P. rubicunda*	6350	171	6300	93	1m	6350	105	66	171	6221	69	6310	75	1.01	m/f	6266	72
Cat	*Trachypithecus cristatus*	6850	433	8350	64	2f	6145	224	149	372	6060	58	6728	62	1.11	f	6060	58
Cat	*T. obscurus*	7580	315	7400	68	1f/2m	7170	182	133	314	6765	59	7347	64	1.09	m/f	7056	62
Cat	*Symphalangus syndactylus*	9300	490	10750	122	1f	11340	146	390	536	11295	124	11453	126	1.01	m/f	11374	125
Cat	*Hylobates pileatus*					1f	7260	56	238	294	5470	85	5500	95	1.01	m/f	5485	90
Cat	*Hylobates lar**	5200	188	5500	108													
Cat	*Pongo pygmaeus*	64819	1591	53000	413	1f	56250	185	1095	1280	36948	338	80643	413	2.18	m/f	58796	375
Cat	*Gorilla gorilla*					1m	236000	595	4396	4991	71500	434	141500	528	1.98	m	141500	528
Cat	*Homo sapiens***	60800	1107	65000	1300													

All values are in grams. Stre: strepsirrhine, Pla: platyrrhines, Cat: catarrhines, BM: body mass, DT: digestive tract, St: stomach, Int: intestines, N_{ind}: number of individuals used in the average, Dim: index of sexual dimorphism in body mass (BM_{male}/BM_{female}). m/f: average of male and female values.

* *Hylobates lar*: excluded because the specimens were "fixed", which may influence organ masses.

** Testing the Expensive Tissue Hypothesis as a general pattern to explain the *Pan-Homo* distinction requires that *Homo sapiens* be excluded from the comparison. The *Homo* values in the Aiello and Wheeler sample were taken from Aschoff *et al.* (1971), presumably because the body mass value of the Chivers and Hladik individuals is very low (45 kg).

2.3.2. Results

In contrast to the original results of Aiello and Wheeler (1995), our revised sample did not yield any significantly negative correlations between relative brain mass and one of the relative digestive tract variables or the combined digestive tract mass (see Table 2.2). Results did not differ according to whether phylogenetic information was taken into account or not, and whether the two strepsirrhine species were included or not.

Table 2.2: Correlations between brain mass residuals and the residuals of mass of stomach, intestines and digestive tract (sum of stomach and intestines).

		A: dataset from Aiello and Wheeler, excluding *Homo sapiens*			**B: revised dataset**						
		Digestive tract mass			**Stomach mass**			**Intestine mass**		**Digestive tract mas**	
	Sample	N	λ	p-value	N	λ	p-value	λ	p-value	λ	p-value
PGLS	Primates				25	0	0.24 (-)	0	0.17 (+)	0	0.86 (+)
	Anthropoidea	17	1	**0.0001 (-)**	23	0	0.23 (-)	0	0.16 (+)	0	0.94 (+)
IC	Primates				25	1	0.82 (-)	1	0.90 (+)	1	0.93 (-)
	Anthropoidea	17	1	**0.0001 (-)**	23	1	0.91 (-)	1	0.65 (+)	1	0.83 (+)
GLM	Primates				25	0	0.24 (-)	0	0.17 (+)	0	0.86 (+)
	Anthropoidea	17	0	**0.004 (-)**	23	0	0.23 (-)	0	0.16 (+)	0	0.94 (+)

PGLS: phylogenetic least-squares methods, IC: classic independent contrasts (lambda set to 1), GLM: linear model of logged species data (lambda set to 0). (+) indicates a positive correlation, (-) indicates a negative correlation. Significant p-values are shown in bold face.

2.3.3. Discussion

The discrepancy between these results and the originally reported negative correlation is due to a combination of factors. First, the Harvey dataset (Clutton-Brock and Harvey 1980) reported brain size values that were not confirmed for some species in subsequent reports, did not systematically exclude juveniles, and sometimes reported only male values without mentioning this fact. Second, sexual size dimorphism affects body mass more than the size of brain mass (Plavcan 2001), which may confound analyses where sex is not taken into account. Third, brain size data has become available for more platyrrhine species in recent years, reducing the bias toward catarrhine species in the original analysis. Fourth, the criteria for inclusion of the digestive tract specimens were not consistent, regarding the inclusion of juveniles or captives. In conclusion, these differences explain why matching the best available brain and body mass data with the Chivers and Hladik dataset did not yield support for the Expensive Tissue Hypothesis in anthropoid primates.

These results supported the argument that a new collection of organ mass data was required for a consistent test of the Expensive Tissue Hypothesis, both in mammals and primates.

Chapter 3: Effects of preservation methods on organ and body mass

3.1. Objectives

The aim of this experimental setup was to quantify changes in body mass and organ mass that could be caused by the application of cold and alcohol preservation methods in mammals of small body size, using laboratory mice (*Mus musculus*) as a model. By calculating correction factors, we aimed to determine whether we could include morphological measurements of preserved mammalian specimens in our comparative sample without generating more variation error. From previously published comparisons on preservation effects in other groups (see Chapter 1.3.1), we predicted that animals stored in alcohol would show a reduction of total body mass and the mass of internal organs, whereas cold storage would not exert an effect on these variables. After assessing the morphological changes due to preservation, we aimed to calculate correction factors and equations to reconstruct fresh body and organ masses. These factors and equations would be put to test using preserved specimens of yellow-necked mouse (*Apodemus flavicollis)*.

3.2. Material and Methods

Our sample consisted of 40 young-adult outbred laboratory mice (Crl:NMRI(Han), 2 months old, 20 males and 20 females in two batches of 10 males and 10 females) raised under similar conditions at the Institute of Laboratory Animal Science, University of Zurich

(Switzerland). The animals were euthanized with carbon dioxide by qualified staff of the Institute. After euthanization, we divided the specimens in four groups of 5 males and 5 females.

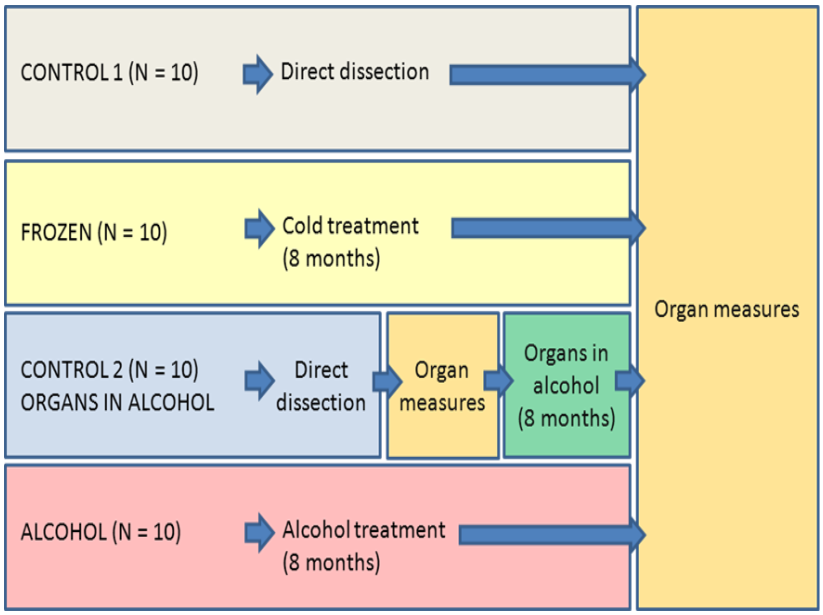


Figure 3.1: Experimental design to measure the effect of cold and alcohol preservation on organ mass

To ensure rapid processing of every batch of specimens, the dissections were conducted in two separate experiments: the first batch was used to compare organ mass of fresh cadavers (*control 1*) to that of specimens kept frozen for 8 months (*cold*), and the second batch was used to compare organ mass of fresh cadavers (*control 2*) to that of specimens (*whole body alcohol*) or individual organs (*organs in alcohol*), both kept for 8 months in 70% alcohol. Within each experiment, 10 individuals (five females and five males) were randomly assigned to each group (see Figure 3.1). We expected that, after the 8 months of treatment, body and organ masses of the individuals in the *alcohol* group would shrink in comparison to those of the *control* and *frozen* groups, meanwhile, no differences would be observed between the *control* and *frozen* groups. In the *organs in alcohol* group, we expected that direct exposition to the preservative would

accelerate diffusion and increase the effect of the treatment in comparison to the *alcohol* group. Note that the groups *control 2* and *organs in alcohol* consist of the same specimens before and after preservation treatment.

I performed all dissections and took all measurements following an identical protocol. Specimens in the *control 1* group (N=10) were weighed, dissected and the mass of the organs was measured immediately after delivery. Organs were initially drained of blood using dry paper and immediately covered by wet paper to avoid desiccation during dissection and until measurement. The heart was drained of blood after opening its chambers with a scalpel. Specimens in the *cold* group (N=10) were weighed, individually put in sealed plastic bags to avoid desiccation, and stored in a freezer at -4 °C for 8 months. Specimens in the *whole body in alcohol* group (N=10) were weighed, 70% alcohol was injected into their abdominal cavity, and they were immersed for 8 months in 70% alcohol in a sealed 5 L plastic container after an identification tag was tied to one of the hind limbs. The container was stored in a dark room at room temperature. Specimens in the *control 2* group were weighed, dissected and organ mass was measured immediately after delivery following the same procedure as in *control 1*. After measurement, the individual organs were then immersed in 70% alcohol in sealed plastic tubes (*organs in alcohol*), and stored for 8 months under the same conditions as the *whole body in alcohol* group. During the whole storage period, alcohol vials were checked regularly every two weeks to avoid liquid leakage. After 8 months, the specimens of the *cold* group and the *whole body in alcohol* group were weighed, dissected and the mass of their organs was measured. The separately kept organs of the *organs in alcohol* group were also weighed. Before body mass measurement and dissection, specimens of the *cold* group were allowed to warm to room

temperature. Animals of the *alcohol* group were drained of excess liquid using paper towels before dissection. Afterwards, dissection in both groups was performed following the same methodology as in the *control* groups. Excess liquid was similarly eliminated in the organs in *the organs in alcohol* group before weighing. Dissections were performed following the same procedure as in the *control* groups.

All mass measurements were taken using a Mettler-Toledo PG 802-S balance. The following organs were measured: heart, lungs, kidneys, spleen, pancreas, liver, stomach, intestines, adipose depots, brain, spinal cord, skin, and the two gastrocnemius muscles. Reproductive organs included the testicles, seminal vesicles, the prostate and the preputial gland for males, and ovaries and uterus for females.

As whole body and organ mass was approximately normally distributed in each group, parametric tests were applied. To compare groups, we used unpaired Student's t-tests, except for comparisons of the same individuals before and after treatment, where a paired t-test was performed.

3.3. Results and correction factors

Mean total body mass and organ masses of all treatment groups are listed in Table 3.1.

Table 3.1: Averages of total body mass and organ mass in the five treatment groups.

	Control 1	*Cold*	*Control 2*	*Organs in alcohol*	*Whole body in alcohol*
Body mass before treatment	38.00 ± 5.59	37.09 ± 5.35	34.98 ± 5.37		35.39 ± 6.26
After treatment		37.06 ± 5.34			27.26 ± 5.25
Kidneys	0.60 ± 0.16	0.52 ± 0.16	0.56 ± 0.17	0.29 ± 0.09	0.21 ± 0.10
Spleen	0.16 ± 0.04	0.13 ± 0.03	0.16 ± 0.03	0.07 ± 0.01	0.04 ± 0.01
Pancreas	0.18 ± 0.04	0.34 ± 0.05	0.19 ± 0.03	0.09 ± 0.03	0.04 ± 0.01
Liver	2.68 ± 0.49	2.10 ± 0.45	2.30 ± 0.48	1.33 ± 0.29	0.84 ± 0.33
Stomach	0.24 ± 0.02	0.19 ± 0.03	0.19 ± 0.03	0.10 ± 0.03	0.06 ± 0.02
Intestines	1.17 ± 0.08	0.93 ± 0.10	1.16 ± 0.10	0.56 ± 0.08	0.48 ± 0.14
Heart	0.24 ± 0.04	0.19 ± 0.04	0.20 ± 0.03	0.09 ± 0.02	0.18 ± 0.04
Lungs	0.28 ± 0.06	0.42 ± 0.05	0.31 ± 0.09	0.21 ± 0.05	0.28 ± 0.06
Male reproductive organs	1.30 ± 0.15	0.99 ± 0.09	1.25 ± 0.19	0.71 ± 0.12	0.62 ± 0.08
Female reproductive organs	0.17 ± 0.04	0.19 ± 0.07	0.21 ± 0.05	0.10 ± 0.04	0.10 ± 0.04
Gastrocnemius muscles	0.40 ± 0.07	0.44 ± 0.12	0.42 ± 0.07	0.18 ± 0.05	0.29 ± 0.07
Adipose depots	5.23 ± 1.21	5.29 ± 1.10	4.86 ± 1.68	4.01 ± 1.43	2.63 ± 0.88
Brain	0.49 ± 0.02	0.46 ± 0.07	0.49 ± 0.01	0.25 ± 0.02	0.28 ± 0.04
Spinal chord	0.09 ± 0.02	0.10 ± 0.04	0.12 ± 0.01	0.09 ± 0.02	0.05 ± 0.01
Skin	5.01 ± 1.47	4.94 ± 1.40	4.62 ± 1.09		3.79 ± 0.75

3.3.1. Total body mass

Results of the comparisons between treatments are shown in Table 3.2. As predicted, specimens in the *cold* group showed no significant loss of body mass after being kept frozen for 8 months (paired t-test: n = 10, t_9 = -1.67, P = 0.13) or in comparison with the *control 1* group (unpaired t-test, n=20, t_{18} = -0.39, P = 0.70). Animals in the *whole body in alcohol* group showed a significant loss of body weight after submersion in 70% alcohol for 8 months (paired t-test: n = 10, t_9 = -14.77, P < 0.0001), or in comparison to the *control 2* group (unpaired t-test, t_{18} = -3.25, P = 0.004). Similar body mass changes were found when sexes were analyzed separately. The quantification of treatment effects and correction factors are reported in later chapter sections.

Table 3.2: Comparison of total body mass before and after cold or alcohol preservation. Significant values are highlighted in bold face.

Analysis	Group	Sex	t-value	P-value
Before treatment vs. after treatment paired t-tests)	Cold	All	-1.67	0.13
		Female	-0.72	0.51
		Male	-1.96	0.12
	Alcohol	All	-14.77	**<0.0001**
		Female	-19.15	**<0.0001**
		Male	-10.1	**0.0003**
Between groups (unpaired t-tests)	Control 1 vs. Cold	All	-0.39	0.70
		Female	-1.17	0.28
		Male	-1.72	0.12
	Control 2 vs. Alcohol	All	-3.25	**0.004**
		Female	-12.14	**<0.0001**
		Male	-6.36	**0.0002**

3.3.2. Organ mass

Results of comparisons between treatments are shown in Table 3.3. Compared with the *control 1* group, some visceral organs of the *cold* group significantly decreased (stomach, intestines, heart and liver) or even increased (pancreas and lungs) in mass. Non-visceral tissues and female reproductive organs did not show any significant difference between these two groups.

In the *whole body in alcohol* group, a significant weight decrease could be observed for most organs, with the exception of heart and lungs, when compared with the *control 2* group. Mass loss was most pronounced in the *organs* in *alcohol* group, where heart and lungs also decreased in mass. Reduction in non-visceral tissue mass was also significant in both alcohol groups. Organ mass changes in both sexes followed the same trend as noticed for the whole sample in all comparisons.

3.3.3. Correction factors

Correction factors were derived as the quotient between the *control 1* group and the *cold* group mean values for those organs with significant mass changes (see Table 3.4).

For the *organs in alcohol* treatment, relationships between organ mass before and after treatment were allometric for most organs. Therefore, we developed linear functions to derive correction formulas (see Table 3.5). Heart, brain and spinal cord masses before and after treatment did not show allometric correlations. For these organs, correction factors were derived as the quotient between the *control 2* group and the *organs in alcohol* mean values. To correct for

body mass, we calculated an allometric relationship between body mass before and after treatment in the *alcohol* group (see Table 3.5).

3.4. Testing the correction factors on yellow-necked wood mice

To test the potential to transfer our correction factors (and equations) to other species, we used measurements from three frozen and three alcohol specimens of *Apodemus flavicollis* (all females). *Apodemus flavicollis* is a muroid species of similar body size range to that of the laboratory mice used in the experimental setup (Silva and Downing 1995). Organ measures in these specimens included all organs measured in mice, with the exception of muscle, pancreas and nervous system, the latter because the intact skulls had to be preserved for osteological collection. We compared body and organ masses with and without applying the correction factors.

The comparison between frozen and alcohol specimens showed significant differences between the two treatment groups in body mass, kidneys, reproductive organs, stomach, intestine, adipose tissue and muscle mass (see Table 3.6 and Figure 3.2). After applying our correction factors, these differences between treatments groups were no longer significant, but significant differences between the two treatment groups appeared for other organs (spleen, heart and lungs).

Table 3.3: Results of the comparison of organ masses before and after treatment. Significant p-values are highlighted in bold face.

		Control 1 vs. *Cold*		*Control 2* vs. *Whole body in alcohol*		*Control 2* vs. *Organs in alcohol*	
Organ	Sex	t-value	P-value	t-value	P-value	t-value	P-value
Kidneys	All	-1.06	0.30	-5.57	**<0.0001**	-10.59	**<.0001**
	Female	-3.31	**0.011**	-12.13	**<0.0001**	-11.78	**0.000**
	Male	-1.91	0.09	-11.59	**<0.0001**	-21.84	**<0.0001**
Spleen	All	-2.01	0.06	-11.47	**<0.0001**	-11.29	**<.0001**
	Female	-3.27	**0.011**	-7.96	**<0.0001**	-8.19	**0.001**
	Male	0.72	0.49	-22.81	**<0.0001**	-12.65	**0.0002**
Pancreas	All	7.89	**0.0001**	-13.27	**<0.0001**	-22.93	**<.0001**
	Female	6.63	**0.0002**	-12.22	**<0.0001**	-17.49	**<0.0001**
	Male	5.16	**0.0009**	-7.60	**<0.0001**	-15.78	**<0.0001**
Liver	All	-2.73	**0.014**	-7.92	**<0.0001**	-15.43	**<.0001**
	Female	-5.19	**0.0008**	-12.90	**<0.0001**	-27.18	**<0.0001**
	Male	-4.28	**0.003**	-12.45	**<0.0001**	-19.94	**<0.0001**
Stomach	All	-3.88	**0.001**	-13.09	**<0.0001**	-14.61	**<.0001**
	Female	-3.11	**0.015**	-9.85	**<0.0001**	-14.70	**0.0001**
	Male	-2.21	0.058	-8.66	**<0.0001**	-14.41	**0.0001**
Intestines	All	-6.14	**0.0001**	-12.37	**<0.0001**	-44.80	**<.0001**
	Female	-5.10	**0.0009**	-11.14	**<0.0001**	-9.49	**0.0007**
	Male	-3.76	**0.006**	-7.24	**<0.0001**	-10.06	**0.0005**
Heart	All	-2.78	**0.013**	-1.16	0.26	-12.12	**<.0001**
	Female	-4.96	**0.001**	-2.72	**0.026**	-30.34	**<0.0001**
	Male	-2.63	**0.030**	-0.73	0.49	-29.59	**<0.0001**
Lungs	All	5.29	**0.0001**	-0.68	0.51	-5.14	**0.001**
	Female	2.26	0.05	-0.50	0.63	-8.26	**0.001**
	Male	6.48	**0.0002**	-0.62	0.55	-11.43	**0.0003**
Muscle	All	1.09	0.29	-4.17	**0.0006**	-23.04	**<.0001**
	Female	0.16	0.88	-3.38	**0.0097**	-3.06	**0.038**
	Male	1.71	0.13	-5.18	**0.0008**	-4.32	**0.012**
Adipose depots	All	-0.61	0.55	-3.56	**0.002**	-8.03	**<.0001**
	Female	0.84	0.43	-8.95	**<0.0001**	-19.30	**<0.0001**
	Male	-1.19	0.27	-2.68	**0.028**	-25.84	**<0.0001**
Brain	All	-1.47	0.16	-17.97	**<0.0001**	-43.70	**<.0001**
	Female	-2.04	0.08	-10.41	**<0.0001**	-7.96	**0.001**
	Male	0.19	0.85	-15.18	**<0.0001**	-4.79	**0.009**
Spinal cord	All	0.51	0.62	-14.42	**<0.0001**	-4.58	**0.001**
	Female	-0.15	0.88	-11.38	**<0.0001**	-30.28	**<0.0001**
	Male	1.14	0.29	-8.55	**<0.0001**	-29.75	**<0.0001**
Skin	All	-0.11	0.92	-1.98	0.06		
	Female	0.44	0.67	-5.19	**0.0008**		
	Male	-0.36	0.73	-4.68	**0.002**		
Reproductive organs	All	NA	NA	NA	NA	NA	NA
	Female	0.59	0.57	-4.21	**0.003**	-14.70	**0.0001**
	Male	-3.93	**0.006**	-6.69	**0.0002**	-14.41	**0.0001**

Table 3.4: Equations for the correction of organ mass after cold treatment.

	Equation
Pancreas	Fresh mass (g) = 0.520*Frozen mass (g)
Liver	Fresh mass (g) = 1.273*Frozen mass (g)
Stomach	Fresh mass (g) = 1.251*Frozen mass (g)
Intestine	Fresh mass (g) = 1.258*Frozen mass (g)
Heart	Fresh mass (g) = 1.244*Frozen mass (g)
Lungs	Fresh mass (g) = 0.675*Frozen mass (g)

Table 3.5: Equations for the correction of organ mass after alcohol treatment.

	r^2	t	P	Equation
Body mass	0.939	11.13	**<0.0001**	Fresh mass (g) = 3.932 + 1.154*Alcohol mass (g)
Kidneys	0.979	19.12	**<0.0001**	Fresh mass (g) = 0.038 + 1.834*Alcohol mass (g)
Spleen	0.452	2.57	**0.033**	Fresh mass (g) = 0.031 + 1.931*Alcohol mass (g)
Pancreas	0.846	6.63	**0.0002**	Fresh mass (g) = 0.085 + 1.135*Alcohol mass (g)
Liver	0.993	32.93	**<0.0001**	Fresh mass (g) = 0.073 + 1.679*Alcohol mass (g)
Stomach	0.525	2.97	**0.018**	Fresh mass (g) = 0.118 + 0.738*Alcohol mass (g)
Intestines	0.888	7.95	**<0.0001**	Fresh mass (g) = 0.424 + 1.311*Alcohol mass (g)
Heart	0.264	1.69	0.13	Fresh mass (g) = 2.176*Alcohol mass (g)
Lungs	0.641	3.78	**0.005**	Fresh mass (g) = 0.019 + 1.349*Alcohol mass (g)
Muscle	0.896	8.32	**<0.0001**	Fresh mass (g) = 0.148 + 1.523*Alcohol mass (g)
Adipose tissue	0.980	20.01	**<0.0001**	Fresh mass (g) = 0.177 + 1.169*Alcohol mass (g)
Brain	0.070	0.78	0.46	Fresh mass (g) = 1.988*Alcohol mass (g)
Spinal cord	0.000	0.08	0.94	Fresh mass (g) = 1.402*Alcohol mass (g)
Repr. (female)	0.974	10.58	**0.0018**	Fresh mass (g) = 0.063 + 1.429*Alcohol mass (g)
Repr. (male)	0.916	5.73	**0.011**	Fresh mass (g) = 0.181 + 1.510*Alcohol mass (g)

3.5. Discussion

The present study intended to systematically assess the effects of preservation on the mass of the internal organs of the body, such as liver, heart, lungs, etc. in mice. We found an unexpected effect of cold storage on the masses of several organs, although changes in total body mass after freezing were slight. Mass reduction associated with wet treatment was observed, as expected. Loss of mass due to alcohol preservation in mice was similar as in other taxa groups (see Table 3.7). We did not evaluate the effect on organ mass of preservation duration, but studies in other animals indicate that mass changes level off after some months (Vervust *et al*. 2009). As all specimens of our comparative sample were exposed to treatment over long periods of time (> 3 months), we did not need to include the effect of preservation time in our corrections.

To test the applicability of our correction factors to other species, we applied the correction formulas to wild specimens of *Apodemus flavicollis* and found that for most organs, the corrected values of frozen specimens did not differ significantly from those of alcohol specimens. However, the thoracic organs (heart and lungs) and the spleen did differ more than before the correction was applied. We therefore concluded that additional studies are warranted to further investigate these effects, and to test if shrinkage due to preservation may be dependant of body size. At present, we cannot advocate the usage of corrections for the use of alcohol stored animals in comparative morphological studies.

Figure 3.2: Comparison of body mass between treatment groups (A) before and (B) after applying correction factors.

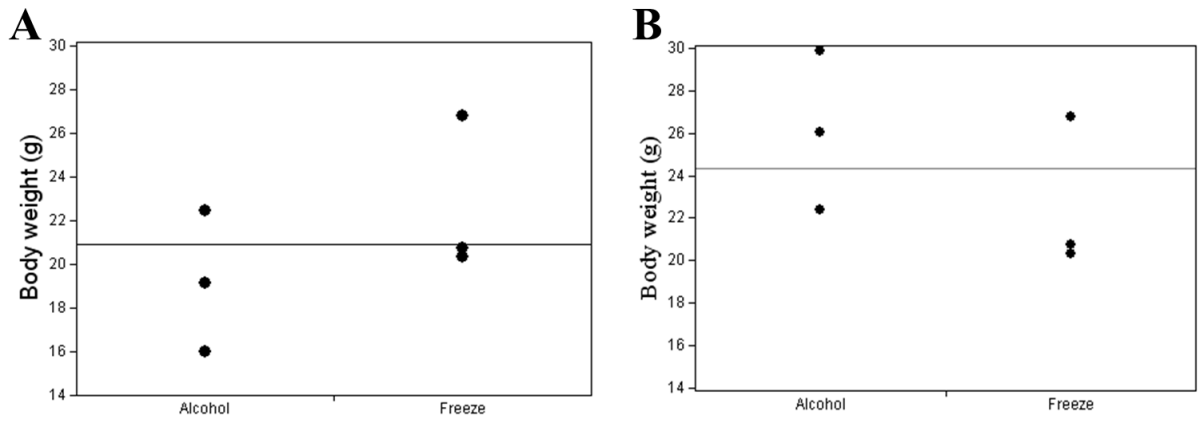


Table 3.6: Body and organ masses of *A. flavicoll*is under cold and alcohol treatment before and after correction.

	Non-corrected			Corrected		
	Cold	Alcohol	p-value	Cold	Alcohol	p-value
Body weight (g)	22.61	19.22	0.30	22.61	26.11	0.31
Kidneys (g)	0.30	0.16	**0.016**	0.30	0.33	0.54
Spleen (g)	0.06	0.06	1.00	0.06	0.15	**0.022**
Liver (g)	0.88	0.72	0.25	1.12	1.29	0.43
Repr. (g)	0.10	0.04	0.07	0.10	0.12	0.46
Stomach (g)	0.26	0.15	**0.020**	0.32	0.23	0.03
Intestines (g)	1.10	0.82	**0.046**	1.38	1.49	0.42
Heart (g)	0.17	0.18	0.62	0.21	0.38	**0.002**
Lungs (g)	0.30	0.43	0.30	0.20	0.60	**0.039**
Adipose tissue (g)	0.58	0.33	**0.0009**	0.58	0.57	0.69
Skin (g)	2.82	2.58	0.73	2.82	3.15	0.69

Table 3.7: Compilation of species with recorded changes in body mass (BM) after alcohol treatment. Values of %BM reflect remaining percentage of body mass after treatment.

Group	Species	Age	Duration	%BM	Authors
Invertebrata	*Heteromastus diversicolor*	adult	90 days	86.1	Wetzel *et al.* 2005
	Heteromastus filiformes	adult	90 days	70.7	Wetzel *et al.* 2005
Anthropoda	*Corophium sp.*	adult	90 days	63.2	Wetzel *et al.* 2005
	Gammarus sp.	adult	90 days	73.3	Wetzel *et al.* 2005
Fish	*Oncorhynchus nerka*	juvenile	70 days	78.7	Shields and Carlson 1996
Reptilia	*Iguana iguana*	juvenile	60 days	86.6	Vervust *et al.* 2009
Mammalia	*Mus musculus*	adults	8 months	79.9	this study
		adult females	8 months	73.1	this study
		adult males	8 months	80.5	this study

3.6. Summary

Our study demonstrated that, if mammal specimens of different preservation methods are to be included in a comparative study using body or organ masses, the usage of a correction formula is required, but more detailed experiments are needed to validate these formulas. For our comparative sample, we concluded that data collected from alcohol individuals should not be included in the analyses to test the Expensive Tissue Hypothesis. Although collection of data from wet individuals was done when available, only frozen specimens were included in the final dataset. We based this decision on the fact that A) changes in the mass of single organs of frozen specimens compared to fresh specimens were less pronounced, amounting to ca. 10-20% in most visceral organs; and B) we had access to a large number of frozen individuals in comparison to fresh or frozen individuals.

Chapter 4: Methods

4.1. Power analysis

To test the Expensive Tissue Hypothesis in mammals, we needed to know how many species were necessary to obtain results with enough statistical power. The power of a test is the probability of rejecting the null hypothesis when the alternative hypothesis is true. Power analyses are required to estimate the sample size needed to adequately test a hypothesis (Sokal and Rohlf 1981). This issue is especially important for results that report the absence of a correlation.

To estimate the minimum sample size needed to detect a correlation between brain size and gut mass in mammals, we used the largest independent dataset available in the literature (Pitts and Bullard 1968, described in the section 2.1), which contains matching gut and central nervous system (CNS) mass data of 39 mammalian species. We assumed that CNS mass, which includes both brain mass and the mass of the spinal cord, was isometrically related to brain mass in mammals. For the power analyses, we did a multiple regression analysis with CNS mass as response variable, gut mass as effect, and fat-free body mass as a covariate to control for the influence of body size.

In this multiple regression, gut mass had a standard error of the residual error sigma of 0.343, and a raw effect size delta of 0.096. We concluded that, for a level of significance of alpha =0.05, the sample size to achieve a power of 0.8 had to be of 103 species. We therefore aimed at collecting data from more than 100 mammal species.

4.2. Morphological data collection

4.2.1. Aims

For the evaluation of the Expensive Tissue and the Energy Trade-off Hypothesis, we intended to collect organ measurements directly from cadaver specimens (Aiello *et al*. 2001). During a two years period, we tracked dead specimens and measured "expensive tissues", i.e. brain, heart, kidneys, liver and digestive tract (Aiello and Wheeler 1995), and other less expensive tissues (i.e. lungs, spleen, pancreas, reproductive organs, and adipose depots), and took a crude measure for skeletal tissue and bone mass (see below).

At the beginning of this study, we aimed to build a dataset of at least 100 species, using intact cadavers which could fulfill the following criteria:

- Fresh, frozen or wet individuals
- Adults (if available, 3 females and 3 males per species, max. 10 individuals)
- Females that were not visibly pregnant
- Non-emaciated individuals (no animals that died of long-term illness)
- If available, species (or genera) with known basal metabolic rates (BMR) would be preferred.

4.2.2. Sources

A main part of our sample was obtained from museums that allowed us to access to specimens that were stored to be processed and included in their collections. Institutions engaged in the recuperation of local fauna, zoological gardens, or hunters also donated some specimens.

Cadavers were obtained from the following institutions and donators:

- Naturhistorisches Museum Basel, Switzerland (contact: Dr. Raffael Winkler)
- Naturhistorisches Museum Fribourg, Switzerland (contact: Dr. Michel Beaud)
- Muséum d'Histoire Naturelle Genève, Switzerland (contact: Dr. Manuel Ruedi)
- Muséum d'Histoire Naturelle Neuchâtel, Switzerland (contact: Dr. Blaise Mulhauser and Martin Zimmerli)
- Zoo Zürich, Switzerland (contact: Dr. Robert Zingg)
- Knies Kinderzoo, Switzerland (contact: Kurt Müller)
- Igelzentrum Zürich, Switzerland (contact: Annekäthi Frei)
- Wildpark Langerberg, Switzerland
- Naturhistorisches Museum Mainz, Germany (contact: Dr. Carsten Renker and Uwe Hildebrand)
- Stattliches Museum für Naturkunde Stuttgart, Germany (contact: Dr. Doris Möricke)
- Zoo Antwerpen, Holland (contact: Dr. Francis Vercammen)
- Royal Museum of African Fauna Tervuren, Belgium (contact: Dr. Emmanuel Gilissen)
- National Museums of Scotland, Great Britain (contact: Dr. Andrew Kitchener)
- Hungarian Natural History Museum, Hungary (contact: Dr. Gabor Csorba and Dr. Laszlo Peregovits)
- Field Museum of Natural History, USA (contact: Dr. William Stanley and Dr. Lawrence Heaney)
- Dr. Carsten Schradin – Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Switzerland
- Dr. Anna Lindholm – Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Switzerland
- Dr. Irmgard Amrein – Institute of Anatomy, University of Zurich, SwitzerlandDr. Marcus Clauss – Clinic for Zoo Animals, Exotic Pets and Wildlife, University of Zurich, Switzerland
- Dr. Alexander Schweiger – Institute of Parasitology, University of Zurich, Switzerland
- Heinz Galli – Anthropological Institute and Museum, University of Zurich, Switzerland
- Wilhelm & Helga Clemens, and Elke Kissel, Germany

4.2.3. Dissection protocol

The dissection protocol was developed in collaboration with Prof. Felix Ehrensperger and colleagues, in the Pathology Department of the Vetsuisse Faculty, University of Zurich, Switzerland.

4.2.3.1. Before dissection

Information about sex, age, origin, time and cause of death of the specimens was to be collected when available. In museums, dissections were performed under the supervision of the museum staff, who performed the more delicate skinning and bone preparation if the specimens were to be prepared for their collections. Donated animals were dissected in the preparation laboratory of the Anthropological Institute in the Irchel Campus of the University of Zurich, Switzerland. In the National Museums of Scotland, the specimens were prepared for dissection by Georg Hantke by removing the bulk of all visceral organs, and keeping them frozen in a sealed plastic bag until I performed the dissections *in situ*. In these specimens, measurements of adipose depots referred to abdominal depots only.

4.2.3.2. Dissection

Frozen specimens were allowed to defreeze (2 h for small specimens, overnight for large specimens) and dried, if wet, externally before weighing. The total body mass was weighed when the body temperature equaled ambient temperature.

The specimens were skinned and the skin was weighed. During dissection, visible subcutaneous accumulations of adipose depots were continuously removed and put in a separate

container. After skinning, the carcass was laid on its back on the dissection surface to facilitate access to the organs.

To avoid desiccation, the organs were put onto wet paper towels immediately after removal. For weighing, individual organs were put onto small sheets of aluminum (small specimens) or into plastic containers (larger specimens). The weight of the aluminum or the container was subsequently subtracted from the measurements. All weights were taken in grams.

4.2.3.2.1. The abdominal cavity

The testes were removed in male specimens. Then, the abdominal cavity was opened with a ventral cut from sternum to pubis and two transverse cuts along the distal end of the rib cage. The spleen was removed. The small intestine was shifted aside. In females, sexual organs were checked at this point to assess reproductive state. The kidneys and additional male sexual structures (seminal vesicles) were cut off and removed. The adrenal glands were separated from the kidneys. The digestive tract was removed cutting the esophagus at the level of the diaphragm and cutting the anus and, in females, the vulva. The female sexual organs were removed. The liver and pancreas were removed. The digestive tract (stomach and intestines) was cleaned from connective and adipose tissue, arrayed on millimeter paper and photographed. Stomach, ileum, caecum and colon were separated, weighed a first time, cut open to remove contents, washed, dried with a paper towel to remove excess water and weighed empty a second time. Digestive tract content was calculated subtracting the second digestive tract measures from the first. The other abdominal organs were weighed. Intestine mass was defined as the sum of ileum, caecum and colon mass. Digestive tract mass was defined as the sum of stomach and intestine mass.

4.2.3.2.2. The thoracic cavity

The diaphragm was cut open following the inner rib cage line. Trachea and esophagus were cut above the sternum, and connective tissue was loosened to allow the thoracic organs to be pulled out distally. The heart was separated, opened, and the chambers were cleaned of blood coagulations by rinsing. The lungs were separated by cutting the bronchi, separating them from the trachea. The thoracic organs were weighed.

4.2.3.2.3. Carcass

Adipose depots were removed from the walls of the abdominal and thoracic cavities. Excess blood was removed with sponges and paper towels. The remaining carcass and the total adipose depots were weighed, sealed in a bag and stored in the freezing chamber.

4.2.3.3. After dissection

4.2.3.3.1. Skeletal measures

Some weeks to months later, the specimens were defrosted, skeletonized, and sometimes degreased, and the bones were dried and weighed. The cranial capacity (endocraneal volume, ECV) was determined using the seed filling method (Isler *et al*. 2008), and converted into an estimate of brain mass by multiplying the volume by 1.036 (Rehkamper *et al*. 1991). A proxy of skeletal tissue mass was obtained by subtracting brain mass and bone mass from remaining carcass mass. However, our skeletal tissue and bone mass measures could not be considered fully reliable and were not analyzed.

4.2.3.3.2. Dry masses

Abdominal and thoracic organs were put in an oven at 150 °C for desiccation. Dry masses were recorded. This part of the protocol was only performed in animals donated for the study (in the preparation laboratory of the Anthropological Institute at the University of Zurich) or in those museums where an oven was available (Muséum d'Histoire Naturelle Neuchâtel and Hungarian Natural History Museum).

4.2.3.3.3. Biological waste

After dissection, all biological material was processed as required by the "Entsorgungsplan für biologisch kontaminierte Abfälle" of the University of Zurich. When the dissections were performed in other institutions, it was disposed of according to the local regulations.

4.3. Dataset compilation

4.3.1. Dataset from dissections

In total, 454 specimens of 133 species were dissected. For further analyses, we redefined and applied the criteria to exclude specimens as follows:

- *Wet (alcohol preserved)* individuals: As consequence of the preservation test (Chapter 3), we excluded individuals preserved in alcohol from the analyses. Except for one specimen (*Sus scrofa*), which was dissected soon after death, all specimens in our sample were preserved by freezing. Therefore, correction factors (cf. Section 3.3.3) were not applied.

- *Juveniles and subadults*: If noted in the records, the age of the animal was compared to the age at first reproduction from Isler's compilation of mammal life history data (Isler, pers. comm.). For animals without records, adulthood of the specimen was determined from body mass and maturity of the sexual organs.
- *Emaciated individuals*: Emaciation was inferred if the body mass of the specimen was less than 75% of the average mass for same-sex adults of the species, using Isler *et al.* (2008) as reference.
- *Pregnant females*: Animals in an advanced state of pregnancy were not considered for dissection. Early stages of pregnancy were not detected until the opening of the abdominal cavity. In these cases (3 specimens of *Vulpes vulpes*, one of *Tamias striatus* and one of *Sciurus niger*), measurements were taken for future study, but specimens were not included in the analyses.
- *Visible damage and pathologies of the organs*: Several animals showed internal macroscopic pathologies, such as tumors. Also, many animals collected in road kills sites had damaged organs. In both cases, an approximate measure for the damaged or pathological organ was taken, but the specimen was excluded from the analyses.
- *Incomplete measurements*: Specimens were excluded from analyses, if they had a broken neurocranium (as endocranial volume could not be measured then), body mass prior to dissection was unknown or some relevant organ was missing.
- *Non-identified species*: A few specimens could be attributed to a specific family or genus, but not to a species. In these cases, animals were excluded from analyses.

- *Total adipose depot vs. abdominal adipose depots*: If we had specimens of the same species with either total adipose depot mass and or abdominal adipose depots (see below), only those specimens with total adipose depot mass were included in the calculation of the species averages and in the analyses.

After applying all these criteria, our final sample included 191 specimens from 100 species, which represented ten mammalian orders (see Table 4.1 and Supplementary Information Disk, Table 1A). Species values were obtained by calculating the average of both male and female specimens of the respective species.

The composition of our dataset in relation to the total currently recognized numbers of mammal species and families is shown in Table 4.2. Although our dataset covered less than 2% of all mammal species, about a quarter of all mammal families were currently represented in our sample. However, Carnivora, Rodentia and Primates were overrepresented at the species level in our sample, whereas many small orders were not represented.

While phylogenetic methods are quite suitable to accommodate grade shifts in the data, an imbalance in the sample in combination with a different relationship within a speciose taxon from that in other taxa may still affect the results. One option to control for a possible bias was to examine large groups separately, which was done here for Primates (N=23 species), Rodentia (N=29 species), and Carnivora (N=28 species). Another option was to resample a better-balanced subset of the data, accepting a reduction in sample size. We did this by selecting one species from each subfamily according to data quality (sample size, wild > captive, females > males, total

adipose depots measured). Our final subfamily sample included 51 species (see Supplementary Information Disc, Table 1G).

Table 4.1: Composition of the organ mass dataset. Numbers of mammalian species are taken from Wilson and Reeder (2005).

	Species			**Families**		
	All	in our sample	coverage [%]	All	in our sample	coverage [%]
Marsupialia						
Didelphimorphia	87	1	1.1	1	1	100.0
Diprotodontia	143	3	2.1	11	3	27.3
other marsupials	101	0	0	9	0	0
Monotremata	5	0	0	2	0	0
Placentalia						
Artiodactyla	240	3	1.3	10	3	30.0
Carnivora	286	28	9.8	15	9	60.0
Chiroptera	1116	3	0.3	18	1	5.6
Erinaceomorpha	24	1	4.2	1	1	100.0
Lagomorpha	92	2	2.2	3	1	33.3
Primates	376	23	6.1	15	8	53.3
Rodentia	2277	29	1.3	33	11	33.3
Scandentia	20	1	5.0	2	1	50.0
Soricimorpha	428	6	1.4	4	2	50.0
other mammals	221	0	0	29	0	0
Total	5195	100	1.9	124	30	24.2

4.3.2. Adipose depots

For 45 species in our sample, adipose depots of the whole body were measured as described in the dissection protocol. For the other 55 species, only abdominal adipose depots were available. Abdominal adipose depots were defined here as the sum of the accumulations of intra-abdominal adipose tissue (see Figure 4.1).

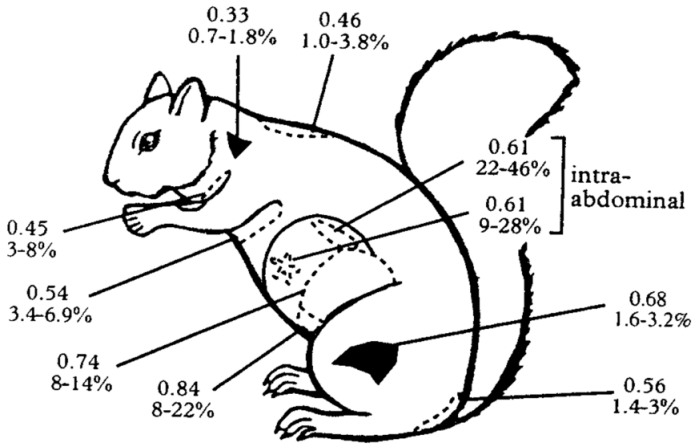


Figure 4.1: Distribution of adipose tissue in the grey squirrel (*Sciurus carolinensis*). Body mass: 0.38-0.67 kg, dissectible adipose tissue: 3.1-14.4% body mass (Pond 1998).

For these 55 species, we used abdominal adipose depot mass to estimate total adipose depot mass, developing two different methods to calculate total adipose depot mass after abdominal adipose depot mass.

The first method consisted of scaling the abdominal fat mass with a scaling factor of 3.419, which was derived from a comparison of the two measurements (total adipose depot mass and abdominal adipose depot mass) for all individuals for which body mass and one of the two fat measurements was available (N=292). As the interaction of measurement type*body mass did not have a significant effect on fat mass, a common slope of 1.0401 was fitted to both subsamples (ln(abdominal fat) = -4.577528 + 1.0401*ln(body mass), N=104; ln(total fat) = -3.348186 + 1.0401*ln(body mass), N=188), yielding a scaling factor of exp(-4.577528-3.348186)=3.419 (see Figure 4.2).

The second scaling was calculated using the correlation between abdominal adipose depots and total adipose depots in 9 specimens for which we had both measurements (*Rattus*

norvegicus, two *Mus musculus*, *Glis glis*, *Martes foina*, *Mephitis mephitis*, *Vulpes vulpes*, *Saimiri sciureus* and *Macaca fuscata*). A least-squares regression (see Figure 4.3) between total adipose depot mass and abdominal depot mass yielded the following prediction equation:

ln(total adipose depots) = 0.564+1.047*ln(abdominal adipose depots)

The obtained values of total adipose depot mass after applying both scaling corrections were very similar. For further analyses, we used the estimation of total adipose depot mass from the first scaling method. We additionally ran two sets of analyses: one with the sample of 100 species with directly measured and estimated adipose depot values, and another with the sample of 45 species with *only* directly measured adipose depot values (see Supplementary Information Disk, Table 2A).

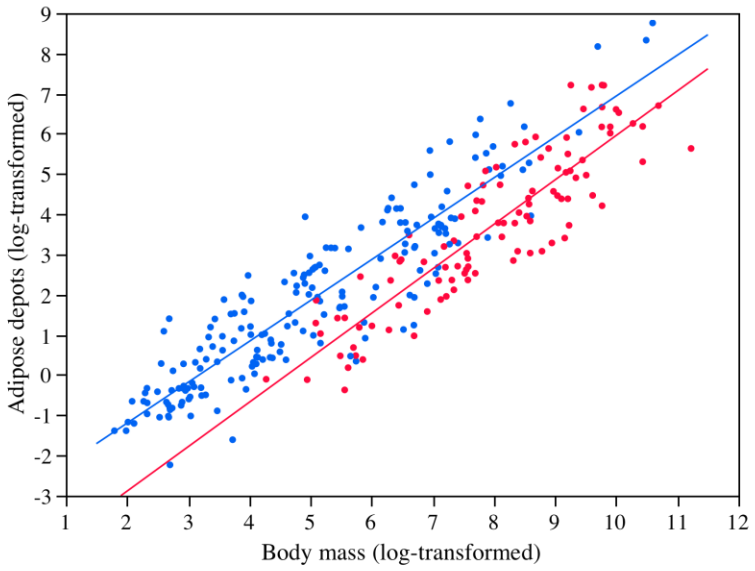


Figure 4.2: Regression of adipose depots mass on body mass for 292 specimens. Blue symbols: specimens with total adipose depots. Red symbols: specimens with abdominal adipose depots.

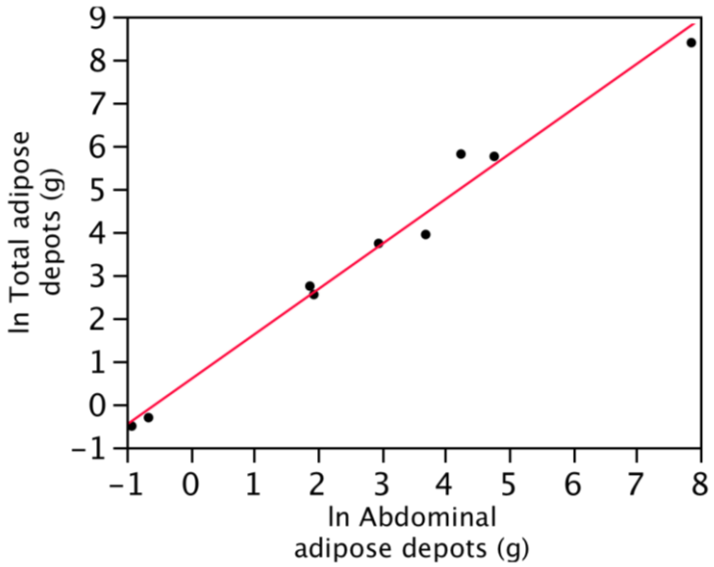


Figure 4.3: Regression of abdominal adipose depots vs. total adipose depots (N=9 specimens, r^2=0.982, P<0.0001).

4.3.3. Sex, provenience and habitat

Averages for *sex*, *provenience* and *habitat* subsets were calculated for the 100 species of our dataset (see Supplementary Information Disk, Tables 1B-F and 2B-F). Sample sizes for each group are detailed in Table 4.2. In the *provenience* samples, two species without concrete details about origin were excluded (*Rattus norvegicus* and *Acomys minous*). For the *habitat* subsamples, we excluded species with both tropical and temperate distribution (N=5).

Table 4.2: Dataset composition (number of species) for each group in the samples of 100 and 45 species.

Effect	Group	Sample (N=100)	Sample (N=45)
Habitat	Temperate	52	39
	Tropical	43	4
Captivity	Captive	59	8
	Wild	39	35
Sex	Male	69	33
	Females	57	30
	Wild females	28	25

4.3.4. Combined dataset including morphological data from the literature

Our sample was biased towards small and middle sized species. Therefore we tried to control for this bias by combining our dataset with sources from the literature (as seen in Chapter 2). Sources were only considered if their methodology was compatible with our dissection protocol, and matching brain and visceral organ mass data was given. Data from the literature did not include spleen mass or adipose depots. As a proxy of the amount of non-visceral tissue, we defined a new variable (*rest)*, defined as total body mass minus the mass of the brain and the visceral organs.

The inclusion of species from the literature yielded a combined sample of 131 species, from 12 mammalian orders (see Supplementary Information Disk, Table 3), and effectively reduced the previous bias towards small and medium-sized species observed in our sample of 100 species. Most of the included species were taken from Crile and Quiring (1940), which has a good representation of large-sized species. Morphological measurements of single species were taken from other publications (*Elephas maximus*: Shoshani 1982; *Phocoena phocoena*: McLellan *et al*. 2002). We also included here measurements of wild *Sus scrofa*, *Pan paniscus* and *Acinonyx jubatus*, which were obtained directly from our dissections, but had not been included in the main dataset of 100 species, because specific organ measurements were missing. The first two species could not be included in the sample of 100 species because no adipose depot measurements were available for the specimens. The third species was not included because spleen mass had not been recorded.

4.3.5. Basal metabolic rates

We took the basal metabolic rate (BMR) data of the species of our sample or of closely related taxa from the recent compilation of Sieg *et al.* (2009), complemented with information from Michel Genoud (pers. comm.). In total, BMR values were available for 64 coincident species of our organ mass sample of 100 species. Values and sources are listed in the Supplementary Information Disk, Table 1A.

Additionally, we also searched the literature for organ metabolic rates, i.e. the metabolic consumption for each separated organ. However, our search showed us that organ metabolic rates were published for only few species, with the additional problem that studies on organ metabolism use different, incompatible methodologies. Therefore, preliminary attempts to analyze organ metabolic consumption rather than organ mass yielded results that were largely identical to those from organ mass alone.

4.4. Controlling for body size

Many physiological traits scale with body size (e.g. Schmidt-Nielsen 1984). In morphological comparative studies, where physiological correlations between different organs are put to test, this body size dependence must be taken into account in the analyses, especially when the sample includes species from very small to very large body size, although there has been some discussion about which variable is the most appropriate to be used as an indicator of body size. Traditionally, we use *whole body mass* for controlling for body size effects in comparative analyses. However, as this measure is highly affected by variation in the size of adipose depots (Pond 1998), we hypothesized here that using this measure to control for body size may have an effect on the correlation between organs: even if two species have a similar *fat-*

free body mass and body composition (organ masses), large adipose depots in one species means that its organs would seem relatively smaller in comparison to those of a species with smaller adipose depots (see Figure 4.4B). Therefore, we expected correlations between organs to be mostly positive if we controlled for *whole body mass* (see Figure 4.4C). This bias was expected to disappear if *fat-free body mass* was used to control for body size effects (see Figure 4.4D).

Measurements of the adipose depots are, however, not commonly available in the literature. Therefore, we could only correct for this bias when data on this variable was available.

In the combined sample of 131 species, which included species with no recorded adipose depot mass, we only controlled for *whole body mass*, but not for *fat-free body mass*, to test the correlations between organs.

For the sample of 100 species, measurements of adipose depots were available as direct measurement (N=45) or scaled from abdominal fat mass (N=55, see Section 4.3.2). We used our approximation for total adipose depot mass to calculate *fat-free body mass* (*whole body mass* minus adipose depot mass). Analyses in this sample were done controlling for both *whole body mass* and *fat-free body mass*, to compare the reliability of both body size variables. Additionally, we ran the analyses using the sample of 100 species and the subsample with directly measured adipose depot mass of 45 species to see if our approximation for total adipose depot mass gave consistent results.

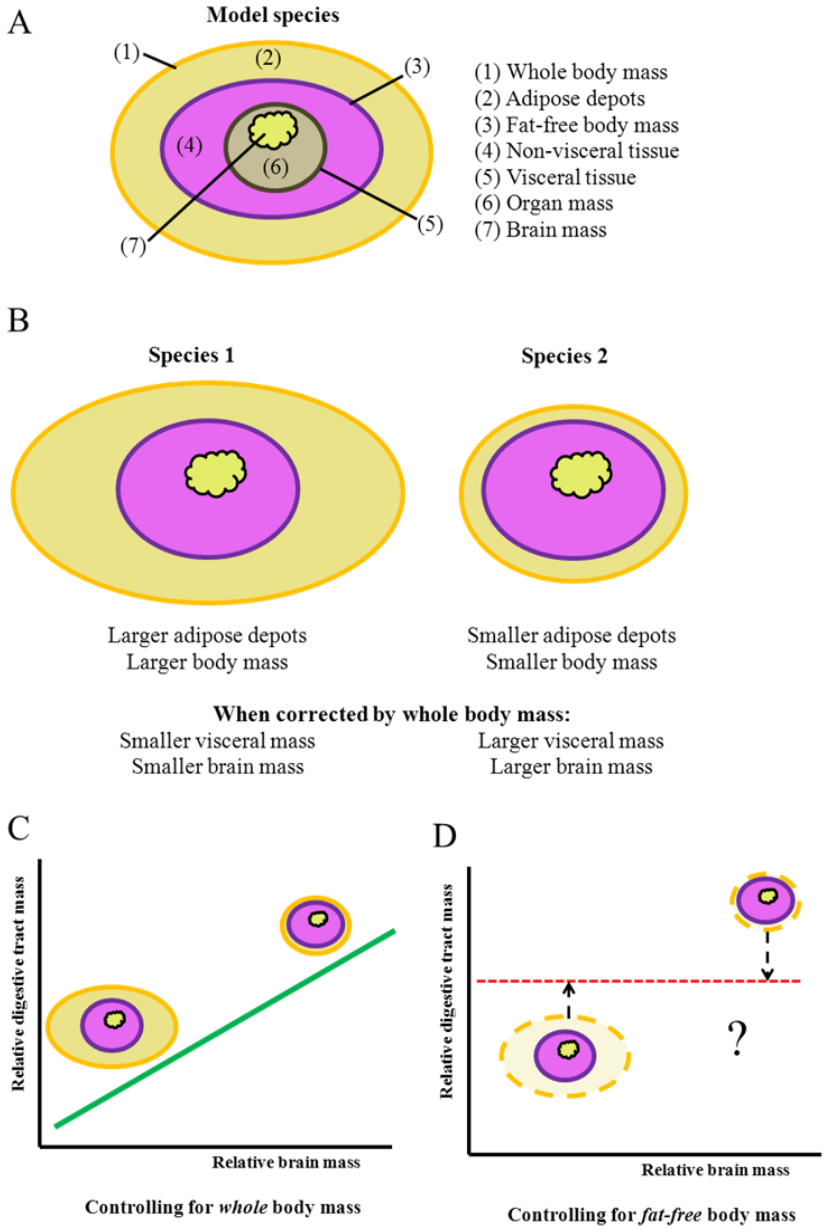


Figure 4.4: Why it is essential to control for body size by using fat-free body mass. A species is depicted by its body composition (A). Two species may have the same fat-free body mass, but different amounts of adipose depots (B). Controlling for whole body mass yields positive correlations between organ masses (C). Only controlling for fat-free body mass allows detecting a possible trade-off between the sizes of different organs (D).

4.5. Phylogenetic analyses

4.5.1. Construction of the phylogenetic trees

Phylogenetic relationships between species were based on the supertree of Bininda-Emonds *et al.* (2007), which included all species in our sample. As the phylogenetic generalized least-squares method (PGLS) requires a completely resolved phylogeny, polytomies were resolved using various sources. Within Chiroptera, *Lasiurus* and *Nyctalus* are more closely related to each other than to *Lasionycteris* (Volleth and Heller 1994). Within Carnivora, polytomies in the Lutrinae group were resolved using Koepfli *et al.* (2008). Within Rodentia, the phylogeny of Cricetidae follows Robovský *et al*. (2008), the one of Muridae follows Lecompte *et al.* (2008), and the distance between Cricetidae and Muridae was taken from Michaux *et al.* (2001). Within Caviidae, distances were taken from Rowe and Honeycutt (2002) and Veniaminova (2007). Within Artiodactyla, the phylogeny of Antilopinae, Aepycerotinae and Alcelaphinae was taken from Hernández-Fernández and Vrba (2005). Although phylogeny in Primates was resolved, distances within this group were modified following the most recent sources (Arnold *et al*. 2010).

For the analyses, we constructed two phylogenetic trees for the combined sample of 131 species, comprising 12 orders (see Figure 4.5), and the sample of 100 species, comprising 9 orders (see Figure 4.6).

Figure 4.5: Phylogenetic tree for the combined sample (N=131). Distances are given in million years before present.

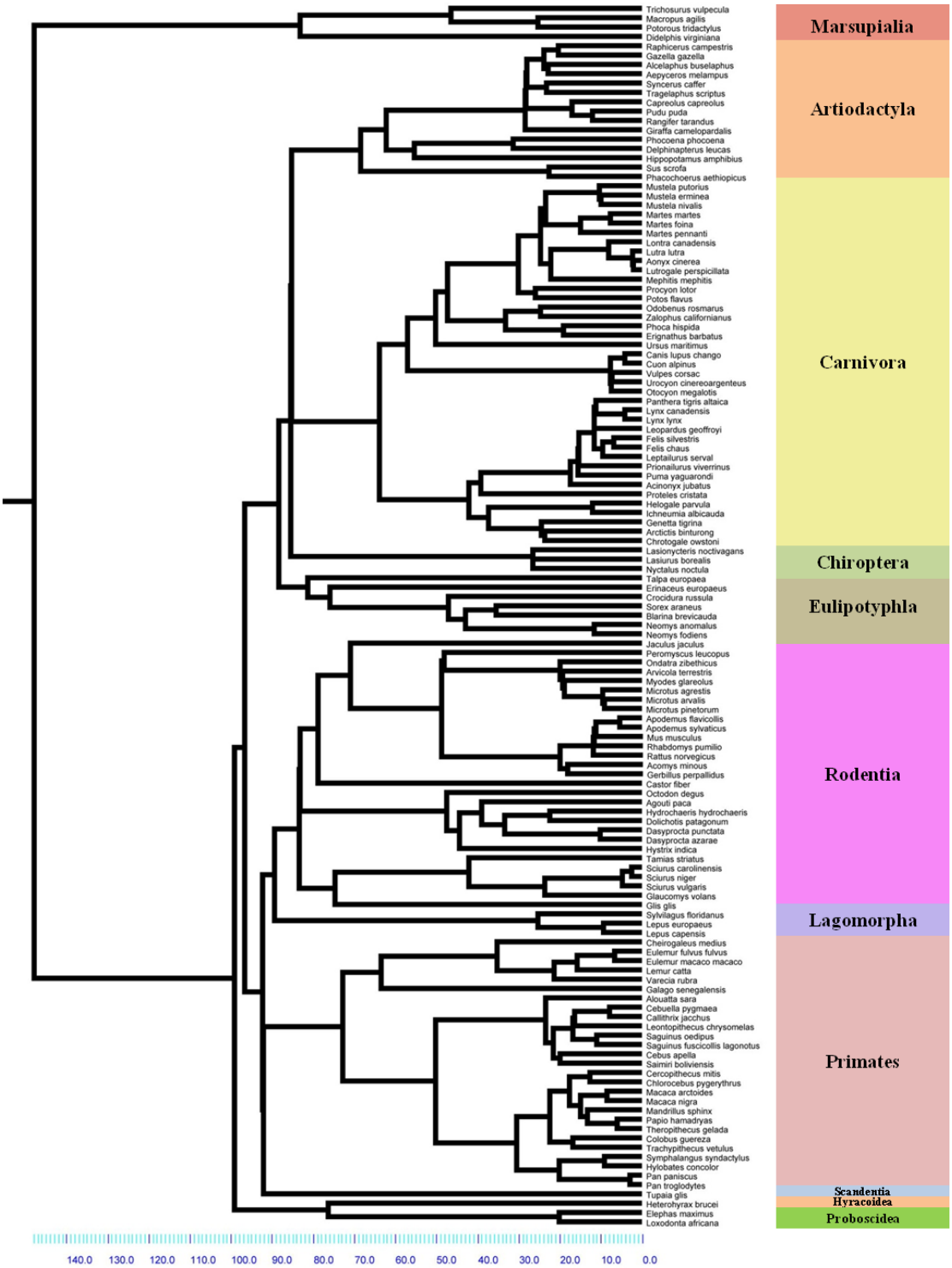
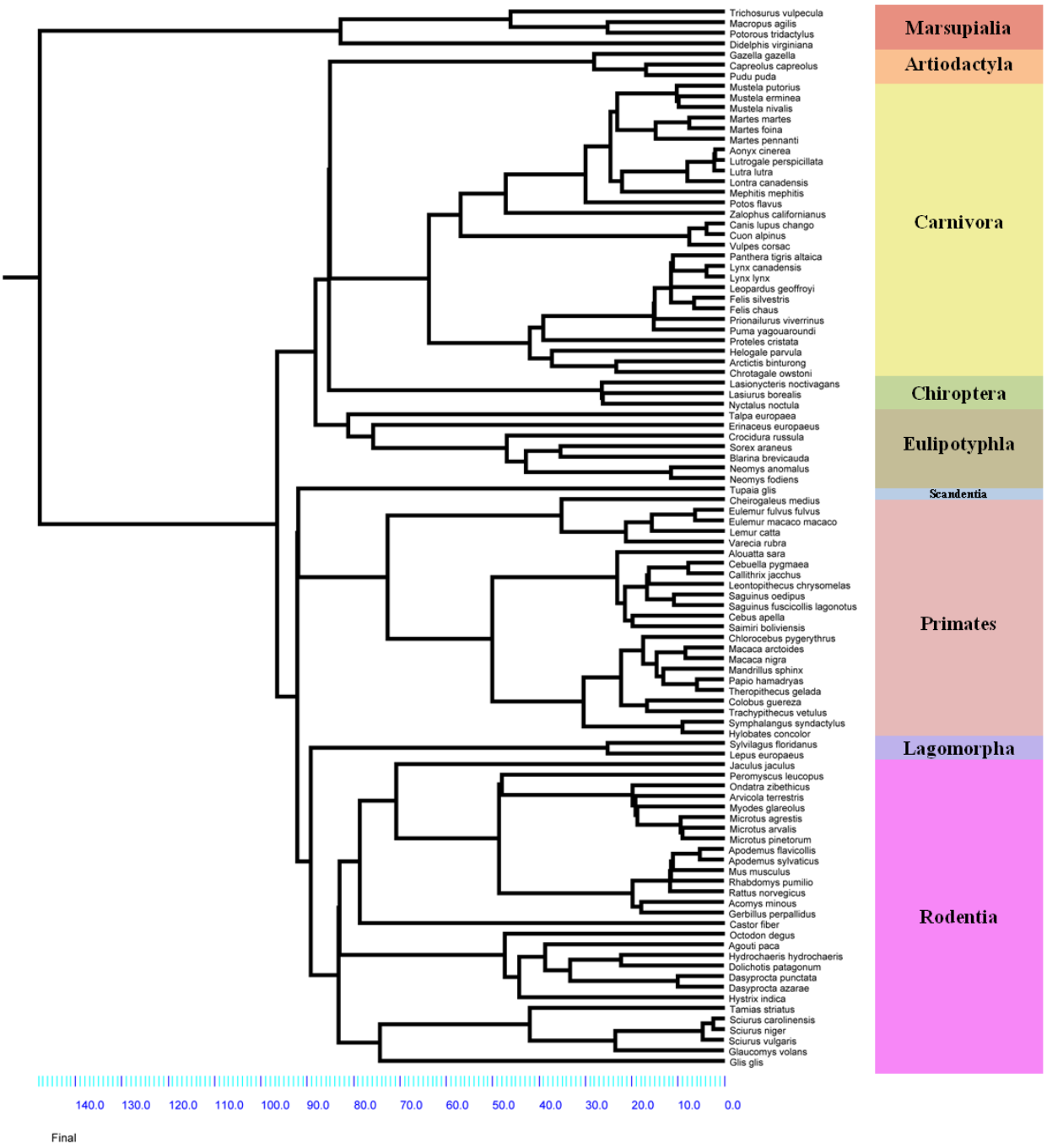


Figure 4.6: Phylogenetic tree for the sample (N=100). Distances are given in million years before present.



4.5.2. Phylogenetic regression methods

Closely related species are expected to be more similar morphologically than distantly related species. This phylogenetic inertia can lead to biased results and spurious relationships, if the similarity is not due to stabilizing selection. Therefore, we needed to control for phylogenetic effects. We used phylogenetic analyses, i.e. analyses that control for these phylogenetic effects, to test for correlations between organs. Traditionally, control for phylogenetic independence has been done calculating independent contrasts (IC). However, in the last years, phylogenetic generalized least-squares (PGLS) regression has become the method of choice, as it allows accommodation for a differential amount of phylogenetic structure in the data.

After all variables were log-transformed, phylogenetic regressions were run using pglm.estLambda in the CAIC (Orme *et al*. 2009) package in R (Team 2010). This function uses the phylogenetic generalized least-squares (PGLS) method, estimating lambda (λ) as an index of the amount of phylogenetic autocorrelation in the data. If lambda is 0, species values are phylogenetically independent and the analysis is equivalent to a species means least-squares regression. If lambda is close to 1, the phylogenetic signal implies that trait evolution has followed Brownian motion, and the analysis is equivalent to the classic method of calculating independent contrasts (IC).

PGLS results were complemented with the results of analyses with lambda set to 0 (raw data) or 1 (classic independent contrasts) using the pic function in the *ape* package (Paradis *et al*. 2004), if the lambda value was not significantly different from either 0 or 1 (italics).

4.5.3. Multiple least-squares regression models

Our models included brain mass as response, body size (*whole body mass*, *fat-free body mass* or *fat-free body mass minus brain mass and organ or tissue mass*, see next section) as covariate, and organ or tissue mass as effect. Organ variables included single visceral organs (heart, lungs, kidneys, liver, digestive tract, and spleen), the sum of all visceral organs, and adipose depots.

To account for differences in the metabolic throughput of the species, we ran additional analyses using basal metabolic rate (BMR) as a proxy. In the above models, we included BMR and the body mass associated to BMR, as covariates. As it has been demonstrated that BMR correlates better with fat-free body mass than with whole body mass (Webb 1981; Nelson *et al*. 1992; Weinsier *et al*. 1992; Johnstone *et al*. 2005), we also alternatively included fat-free body mass (associated to brain and organ mass) as a covariate.

4.5.4. Autocorrelation effects

Another potential problem in the analysis of body composition data is the presence of autocorrelation effects, as the organs are part of the body mass (*whole body mass* or *fat-free body mass*) which is used to control for size effects (Christians 1999).

These effects are most pronounced when the parts comprise a large proportion of the whole body. This is not the case in most of the correlations between brain size and one of the visceral organs in our sample, as these organs count for a small part in body composition. Furthermore, autocorrelation assumes that the total size of a sum of variables is strictly limited and thus constant. In the case of body mass, this assumption is overly conservative, because adding the size of some organ does not need to constrain the size of other organs. Nevertheless, to

validate our results we additionally applied adjusting our estimate of body size by controlling for *fat-free body mass minus brain mass and the respective organ mass* instead of just fat-free body mass.

To control for potential autocorrelation effects in the correlations between brain size and specific tissues, we also we applied compositional data analysis. This method was developed for mathematical geology (Aitchinson 1982), and it has been applied to intraspecific body composition data before (Muldowney *et al*. 2001). It takes into account that *n* parts of a whole are by necessity autocorrelated and transforms the values by projecting them onto a simplex of *n-1* dimensions. We used the function *acomp* for closed compositions in a logistic geometry, following a log-ratio approach and the isometric log-ratio transform *ilr* (Egozcue *et al*. 2003) from the package **compositions** (van den Boogart and Tolosano-Delgado 2008) in R (Team 2010). Thus, from the raw values of brain mass, adipose depots mass and the remaining body mass without brain and adipose depots, we obtained two transformed variables, which are no longer autocorrelated. We then tested the correlation between these two variables with phylogenetic regression using *pglmEstLambda* from the package **CAIC** in R.

4.5.5. Principal components analysis (PCA)

To identify patterns in organ clustering, we ran a principal components analysis (PCA), on the residuals of organ mass vs. fat-free body mass using JMP (SAS 1989-2005). Principal components with Eigenvalues larger than 1 were rotated with Varimax rotation.

Chapter 5: Results

5.1. Correlations between brain size and organ masses

In this section, we present our results on the correlations between brain mass and mass of other organs or tissues for the different samples (100 species, 45 species and 131 species) and for the different variables used to control for body size (*whole body mass*, *fat-free body mass*, and *fat-free body mass minus brain mass and organ mass*).

5.1.1 Sample of 100 species

In this sample, we found several positive correlations between brain mass and the mass of some visceral organs (heart, kidneys, liver and digestive tract), which were dependant on the method of control for body size (see Table 5.1). When controlling for body size using *whole body mass*, some correlations were significantly positive (heart and digestive tract, see Table 5.1a). These positive correlations were observed in mammals and Primates, and in Rodentia (only for heart mass), but not in Carnivora. When controlling for body size using *fat-free body mass* or *fat-free body mass minus brain mass and organ mass*, the positive correlations were no longer significant in all groups except in Primates (see Table 5.1b and c).

On the other hand, a significant negative correlation between brain size and adipose depot mass in mammals was found in all samples, for all three methods to control for body size (see Table 5.1). However, within orders, this correlation was not found in Primates. In Carnivora, it was only present when *whole body mass* was used as a covariate. In Rodentia, the negative trend was close to significance if *fat-free body mass* or *fat-free body mass minus brain mass and organ mass* as a covariate.

Table 5.1: Pair-wise phylogenetic least-squares regression (PGLS) between brain and other organ masses (N=100 species), controlling for a) *whole body mass*, b) *fat-free body mass*, and c) *fat-free body mass minus brain mass and organ mass*.

a)	**Including *whole body mass* as covariate**											
	PGLS						Independent contrasts (λ = 1)			Raw data (λ = 0)		
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	β	t-value	p-value	β	t-value	p-value
Mammals	**N=100**											
Heart	0.905	<0.0001	0.0002	0.22	2.32	**0.022**						
Lungs	0.902	<0.0001	0.0005	0.07	0.67	0.50						
Kidneys	0.901	<0.0001	0.0003	0.09	0.87	0.38						
Liver	0.907	<0.0001	0.0005	0.04	0.47	0.64						
Digestive tract	0.940	<0.0001	0.003	0.22	2.70	**0.008**						
Stomach	0.928	<0.0001	0.002	0.19	2.59	**0.011**						
Intestine	0.938	<0.0001	0.003	0.16	2.31	**0.023**						
Spleen	0.912	<0.0001	0.0010	-0.01	-0.29	0.77						
Visceral organs	0.909	<0.0001	0.0002	0.16	1.42	0.16						
Adipose depots	0.932	<0.0001	0.024	-0.12	-4.00	**0.0001**						
Primates	**N=23**											
Heart	0.445	0.74	0.036	0.63	3.18	**0.005**						
Lungs	0.759	*0.10*	*0.28*	0.45	2.01	0.06	0.60	2.31	**0.032**	0.47	2.16	**0.043**
Kidneys	0.756	0.028	0.070	0.34	1.75	0.10						
Liver	0.737	*0.09*	*0.14*	0.21	1.12	0.28	0.17	0.91	0.38	0.33	1.52	0.14
Digestive tract	1.000	*0.07*	*1.00*	0.49	3.30	**0.004**				0.55	3.06	**0.006**
Stomach	0.887	0.033	0.53	0.17	1.28	0.21						
Intestine	1.000	*0.06*	*1.00*	0.42	3.22	**0.004**				0.45	2.92	**0.009**
Spleen	0.775	0.009	0.36	0.18	1.95	0.07						
Visceral organs	0.734	*0.12*	*0.34*	0.51	2.44	**0.024**	0.53	2.62	**0.016**	0.63	2.77	**0.012**
Adipose depots	0.781	*0.26*	*0.21*	-0.07	-0.91	0.37	-0.06	-1.01	0.32	-0.15	-1.87	0.08
Carnivora	**N=28**											
Heart	0.000	1.00	0.0001	0.06	0.41	0.69						
Lungs	0.000	1.00	0.001	-0.03	-0.17	0.86						
Kidneys	0.000	1.00	0.0002	0.14	0.91	0.37						
Liver	0.000	1.00	0.0002	0.07	0.63	0.53						
Digestive tract	0.000	1.00	0.0006	0.28	1.58	0.13						
Stomach	0.000	1.00	0.0004	0.15	0.96	0.35						
Intestine	0.000	1.00	0.0006	0.26	1.66	0.11						
Spleen	0.000	1.00	0.003	-0.03	-0.55	0.59						
Visceral organs	0.000	1.00	0.0001	0.13	0.63	0.53						
Adipose depots	0.000	1.00	<0.0001	-0.11	-2.09	**0.047**						
Rodentia	**N=29**											
Heart	0.732	0.001	0.0008	0.28	2.31	**0.029**						
Lungs	0.754	0.0006	0.0006	0.05	0.35	0.73						
Kidneys	0.754	0.0009	0.0006	-0.03	-0.17	0.86						
Liver	0.755	0.0007	0.0008	-0.07	-0.43	0.66						
Digestive tract	0.792	0.002	0.005	0.08	0.51	0.61						
Stomach	0.809	0.001	0.026	0.11	0.74	0.46						
Intestine	0.779	0.002	0.002	0.05	0.35	0.73						
Spleen	0.804	0.0001	0.003	-0.08	-1.37	0.18						
Visceral organs	0.762	0.0009	0.0006	0.03	0.12	0.90						
Adipose depots	0.800	<0.0001	0.007	-0.11	-2.67	**0.013**						

Table 5.1 continued:

b)	Including *fat-free body mass* as covariate											
	PGLS						Independent contrasts (λ = 1)			Raw data (λ = 0)		
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	β	t-value	p-value	β	t-value	p-value
Mammals	**N=100**											
Heart	0.918	<0.0001	0.001	0.15	1.51	0.13						
Lungs	0.928	<0.0001	0.010	-0.03	-0.35	0.73						
Kidneys	0.921	<0.0001	0.004	0.01	0.10	0.92						
Liver	0.923	<0.0001	0.005	-0.02	-0.19	0.84						
Digestive tract	0.943	<0.0001	0.007	0.16	1.89	0.06						
Stomach	0.934	<0.0001	0.005	0.15	2.06	**0.042**						
Intestine	0.940	<0.0001	0.007	0.11	1.45	0.15						
Spleen	0.929	<0.0001	0.008	-0.02	-0.53	0.60						
Visceral organs	0.922	<0.0001	0.0020	0.05	0.46	0.64						
Adipose depots	0.938	<0.0001	0.029	-0.07	-2.42	**0.017**						
Primates	**N=23**											
Heart	0.347	1.00	0.033	0.65	2.99	**0.007**						
Lungs	0.703	*0.13*	*0.25*	0.44	1.93	0.07	0.44	1.97	0.06	0.51	1.97	0.06
Kidneys	0.717	*0.05*	*0.69*	0.34	1.86	0.08	0.24	1.27	0.22	0.31	1.46	0.16
Liver	0.664	*0.18*	*0.16*	0.20	1.06	0.30	0.14	0.73	0.48	0.27	1.29	0.21
Digestive tract	1.000	*0.10*	*1.00*	0.48	3.14	**0.005**				0.5	2.79	**0.011**
Stomach	0.857	*0.07*	*0.57*	0.17	1.35	0.19	0.22	1.71	0.10	0.14	1.03	0.31
Intestine	1.000	*0.10*	*1.00*	0.41	2.95	**0.008**				0.41	2.57	**0.018**
Spleen	0.838	0.036	0.29	0.15	1.56	0.13						
Visceral organs	0.663	*0.17*	*0.31*	0.50	2.35	**0.029**	0.51	2.43	**0.025**	0.57	2.46	**0.023**
Adipose depots	0.793	*0.29*	*0.23*	-0.01	-0.10	0.92	-0.02	-0.26	0.80	-0.09	-1.11	0.28
Carnivora	**N=28**											
Heart	0.000	1.00	0.0001	-0.02	-0.12	0.91						
Lungs	0.000	1.00	0.003	-0.16	-1.05	0.31						
Kidneys	0.000	1.00	0.001	0.050	0.33	0.74						
Liver	0.000	1.00	0.0003	-0.01	-0.08	0.93						
Digestive tract	0.000	1.00	0.0004	0.16	0.86	0.40						
Stomach	0.000	1.00	0.0002	0.08	0.55	0.59						
Intestine	0.000	1.00	0.0004	0.15	0.90	0.38						
Spleen	0.000	1.00	0.001	-0.03	-0.52	0.61						
Visceral organs	0.000	1.00	0.0003	-0.09	-0.41	0.69						
Adipose depots	0.000	1.00	<0.0001	-0.04	-0.90	0.38						
Rodentia	**N=29**											
Heart	0.762	0.0004	0.001	0.23	1.90	0.07						
Lungs	0.788	0.0002	0.003	-0.02	-0.16	0.87						
Kidneys	0.776	0.0004	0.001	-0.13	-0.78	0.44						
Liver	0.784	0.0002	0.002	-0.10	-0.70	0.49						
Digestive tract	0.774	0.0038	0.0036	-0.03	-0.18	0.86						
Stomach	0.805	0.0007	0.014	0.05	0.34	0.74						
Intestine	0.769	0.003	0.002	-0.04	-0.29	0.78						
Spleen	0.825	<0.0001	0.008	-0.08	-1.43	0.16						
Visceral organs	0.773	0.0007	0.001	-0.10	-0.48	0.63						
Adipose depots	0.821	<0.0001	0.010	-0.08	-1.97	0.06						

Table 5.1 continued:

c)	**Including *fat-free body mass minus brain mass and organ mass* as covariate**											
	PGLS						Independent contrasts (λ = 1)			Raw data (λ = 0)		
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	β	t-value	p-value	β	t-value	p-value
Mammals	**N=100**											
Heart	0.918	<0.0001	0.001	0.16	1.70	0.09						
Lungs	0.926	<0.0001	0.009	-0.01	-0.08	0.93						
Kidneys	0.921	<0.0001	0.004	0.03	0.30	0.77						
Liver	0.923	<0.0001	0.004	0.02	0.30	0.77						
Digestive tract	0.945	<0.0001	0.008	0.19	2.28	**0.025**						
Stomach	0.936	<0.0001	0.006	0.16	2.27	**0.026**						
Intestine	0.942	<0.0001	0.007	0.13	1.77	0.08						
Spleen	0.929	<0.0001	0.008	-0.02	-0.44	0.66						
Visc. organs	0.923	<0.0001	0.002	0.14	1.35	0.18						
Adipose depots	0.939	<0.0001	0.03	-0.07	-2.39	**0.019**						
Primates	**N=23**											
Heart	0.325	1.00	0.032	0.68	3.12	**0.005**						
Lungs	0.702	*0.13*	*0.28*	0.47	2.07	0.05	0.46	2.09	0.050	0.54	2.12	**0.047**
Kidneys	0.719	0.0495	0.07	0.35	1.94	0.07						
Liver	0.670	*0.17*	*0.17*	0.24	1.27	0.22	0.17	0.91	0.38	0.31	1.50	0.15
Digestive tract	1.000	*0.10*	*1.00*	0.49	3.32	**0.003**				0.53	3.01	**0.007**
Stomach	0.882	*0.07*	*0.66*	0.18	1.47	0.16	0.23	1.80	0.09	0.15	1.14	0.27
Intestine	1.000	*0.09*	*1.00*	0.33	2.59	**0.018**				0.44	2.76	**0.012**
Spleen	0.845	0.034	0.31	0.16	1.61	0.12						
Visc. organs	0.674	*0.16*	*0.35*	0.54	2.80	**0.011**	0.54	2.79	**0.011**	0.61	2.91	**0.009**
Adipose depots	0.805	*0.28*	*0.25*	-0.01	-0.07	0.95	-0.01	-0.24	0.81	-0.09	-1.09	0.29
Carnivora	**N=28**											
Heart	0.000	1.00	0.0005	0.01	0.04	0.96						
Lungs	0.000	1.00	0.014	-0.11	-0.76	0.45						
Kidneys	0.000	1.00	0.007	0.080	0.55	0.59						
Liver	0.000	1.00	0.0010	0.03	0.27	0.79						
Digestive tract	0.000	1.00	0.0007	0.19	1.00	0.33						
Stomach	0.000	1.00	0.0009	0.09	0.58	0.57						
Intestine	0.000	1.00	0.0006	0.17	1.02	0.32						
Spleen	0.000	1.00	0.013	-0.02	-0.44	0.67						
Visc. organs	0.000	1.00	0.002	0.04	0.20	0.85						
Adipose depots	0.000	1.00	0.011	-0.05	-1.02	0.32						
Rodentia	**N=29**											
Heart	0.760	0.0005	0.001	0.24	2.00	0.06						
Lungs	0.786	0.0002	0.003	-0.01	-0.03	0.98						
Kidneys	0.775	0.0005	0.001	-0.12	-0.66	0.51						
Liver	0.783	0.0002	0.002	-0.06	-0.44	0.66						
Digestive tract	0.779	0.0030	0.004	-0.02	0.11	0.91						
Stomach	0.808	0.0006	0.016	0.07	0.45	0.66						
Intestine	0.773	0.002	0.002	-0.01	-0.04	0.97						
Spleen	0.824	<0.0001	0.007	-0.08	-1.39	0.18						
Visc. organs	0.774	0.0006	0.001	0.01	0.05	0.96						
Adipose depots	0.820	<0.0001	0.010	-0.08	-1.95	0.06						

5.1.2. Sample of 45 species with total adipose depots mass

As in the sample of 100 species, we did not find a significant negative correlation between brain mass and the mass of any visceral organs. In comparison to the previous sample, however, significant positive correlations between brain mass and visceral organ mass were less frequent if we controlled for *whole body mass* (only significant for heart mass in rodents, see Table 5.2a). Using *fat-free body mass* and *fat-free body mass minus brain and heart mass*, heart mass still showed a significant correlation with brain size in rodents (see Table 5.2b and c).

Independently of the variable used to control for body size, a significant negative correlation was found between brain mass and adipose depot mass in mammals (see Table 5.2) and in the subset of non-rodents. Like in the sample of 100 species, this correlation persisted in rodents when we controlled for *whole body mass* and *fat-free body mass* (see Table 5.2a and b), but was only a trend when we controlled for *fat-free body mass minus brain mass* (see Table 5.2c).

5.1.3. Sample of 131 species, combined with data from the literature

In this sample, correlations were similar to those obtained from the sample of 100 and 45 species controlling for *whole body mass* (see Table 5.3). Between brain size and the mass of other organs, some positive, but no negative trends were observed. Significant positive correlations were again found for heart mass (in rodents and mammals as a group), and for digestive tract mass and the combined mass of all visceral organs in primates.

Table 5.2: Pair-wise phylogenetic least-squares regression (PGLS) between brain and other organ masses (N=45 species), controlling for a) whole body mass, b) fat-free body mass, and c) fat-free body mass minus brain mass and organ mass.

a)	**Including *whole body mass* as covariate**					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Mammals	**N=45**					
Heart	0.942	<0.0001	0.09	0.33	1.98	0.054
Lungs	0.910	<0.0001	0.009	0.15	0.89	0.38
Kidneys	0.906	<0.0001	0.015	0.20	1.17	0.25
Liver	0.912	<0.0001	0.01	0.12	0.93	0.36
Digestive tract	0.945	<0.0001	0.14	0.25	1.72	0.09
Stomach	0.949	<0.0001	0.22	0.32	2	0.05
Intestine	0.935	<0.0001	0.07	0.17	1.36	0.18
Spleen	0.923	<0.0001	0.035	-0.04	-0.77	0.45
Visceral organs	0.928	<0.0001	0.022	0.33	1.64	0.11
Adipose depots	0.929	<0.0001	0.11	-0.18	-4.46	**<0.0001**
Non-Rodentia	**N=21**					
Heart	1.000	0.002	1.00	0.25	0.94	0.36
Lungs	1.000	0.001	1.00	0.34	1.26	0.22
Kidneys	1.000	0.0006	1.00	0.53	1.77	0.09
Liver	1.000	0.0006	1.00	0.26	1.70	0.11
Digestive tract	0.993	0.003	0.94	0.16	0.6	0.56
Stomach	0.990	0.003	0.91	0.21	0.83	0.41
Intestine	0.991	0.003	0.92	0.10	0.44	0.66
Spleen	0.991	0.003	0.92	0.02	0.26	0.80
Visceral organs	1.000	0.0004	1.00	0.56	1.95	0.07
Adipose depots	0.891	0.0006	0.19	-0.27	-3.68	**0.002**
Rodentia	**N=24**					
Heart	0.604	0.11	0.001	0.60	3.14	**0.005**
Lungs	0.712	0.007	0.001	0.12	0.59	0.56
Kidneys	0.727	0.005	0.001	-0.008	-0.04	0.97
Liver	0.725	0.005	0.002	-0.06	-0.30	0.76
Digestive tract	0.825	0.004	0.036	0.20	1.14	0.27
Stomach	0.845	0.004	0.12	0.27	1.26	0.22
Intestine	0.796	0.004	0.012	0.13	0.86	0.40
Spleen	0.800	0.001	0.011	-0.10	-1.45	0.16
Visceral organs	0.746	0.004	0.002	0.10	0.41	0.69
Adipose depots	0.810	0.0004	0.023	0.80	13.65	**<0.0001**

Table 5.2 continued:

b)	**Including *fat-free body mass* as covariate**					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Mammals	**N=45**					
Heart	0.933	<0.0001	0.05	0.18	1.06	0.29
Lungs	0.918	<0.0001	0.024	0.003	0.02	0.99
Kidneys	0.917	<0.0001	0.02	0.05	0.30	0.77
Liver	0.919	<0.0001	0.019	0.05	0.37	0.71
Digestive tract	0.933	<0.0001	0.08	0.11	0.74	0.46
Stomach	0.936	<0.0001	0.10	0.16	0.99	0.33
Intestine	0.928	<0.0001	0.049	0.07	0.53	0.60
Spleen	0.933	<0.0001	0.06	-0.04	-0.92	0.36
Visceral organs	0.924	<0.0001	0.024	0.12	0.58	0.57
Adipose depots	0.940	<0.0001	0.14	-0.13	-3.52	**0.001**
Non-Rodentia	**N=21**					
Heart	0.987	0.001	0.86	0.04	0.14	0.89
Lungs	0.999	0.001	0.99	0.14	0.55	0.59
Kidneys	1.000	0.0006	1.00	0.32	1.12	0.28
Liver	1.000	0.0005	1.00	0.20	1.33	0.20
Digestive tract	0.981	0.002	0.80	-0.02	-0.09	0.93
Stomach	0.981	0.002	0.81	0.01	0.06	0.96
Intestine	0.981	0.002	0.80	-0.04	-0.14	0.89
Spleen	0.981	0.002	0.80	0.009	0.15	0.88
Visceral organs	1.000	0.0008	1.00	0.35	1.18	0.25
Adipose depots	0.917	0.0004	0.30	-0.18	-2.66	**0.016**
Rodentia	**N=24**					
Heart	0.646	0.07	0.001	0.53	2.65	**0.015**
Lungs	0.762	0.002	0.004	0.01	0.07	0.94
Kidneys	0.755	0.003	0.002	-0.12	-0.62	0.54
Liver	0.764	0.002	0.004	-0.11	-0.63	0.53
Digestive tract	0.799	0.006	0.023	0.08	0.43	0.67
Stomach	0.816	0.004	0.049	0.13	0.65	0.52
Intestine	0.783	0.005	0.01	0.04	0.25	0.80
Spleen	0.826	0.0003	0.023	-0.09	-1.47	0.16
Visceral organs	0.758	0.003	0.002	-0.05	-0.20	0.84
Adipose depots	0.829	0.0002	0.031	-0.09	-2.15	**0.043**

Table 5.2 continued:

c)	**Including *fat-free body mass minus brain mass and organ mass* as covariate**					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Mammals	**N=45**					
Heart	0.945	<0.0001	0.10	0.20	1.23	0.22
Lungs	0.930	<0.0001	0.05	0.01	0.07	0.94
Kidneys	0.930	<0.0001	0.043	0.04	0.22	0.83
Liver	0.930	<0.0001	0.033	0.09	0.82	0.41
Digestive tract	0.936	<0.0001	0.08	0.07	0.45	0.66
Stomach	0.949	<0.0001	0.20	0.16	0.99	0.33
Intestine	0.932	<0.0001	0.05	0.03	0.19	0.85
Spleen	0.939	<0.0001	0.08	-0.03	-0.62	0.54
Visceral organs	0.934	<0.0001	0.041	0.17	0.98	0.33
Adipose depots	0.948	<0.0001	0.20	-0.11	-2.84	**0.007**
Non-Rodentia	**N=24**					
Heart	0.693	0.027	0.002	0.49	2.54	**0.019**
Lungs	0.781	0.002	0.009	0.01	0.50	0.96
Kidneys	0.768	0.002	0.003	-0.14	-0.72	0.48
Liver	0.78	0.001	0.007	-0.08	-0.49	0.63
Digestive tract	0.764	0.016	0.013	-0.01	-0.05	0.96
Stomach	0.824	0.005	0.10	0.11	0.53	0.60
Intestine	0.757	0.01	0.007	-0.03	-0.19	0.86
Spleen	0.827	0.0003	0.021	-0.08	-1.15	0.26
Visceral organs	0.768	0.003	0.004	-0.02	-0.10	0.92
Adipose depots	0.826	0.0003	0.029	-0.08	-1.84	0.08
Rodentia	**N=21**					
Heart	0.984	0.001	0.85	0.08	0.30	0.77
Lungs	0.995	0.001	0.95	0.15	0.57	0.57
Kidneys	1.000	0.0009	0.99	0.26	0.92	0.37
Liver	1.000	0.0004	1.00	0.25	1.77	0.09
Digestive tract	0.971	0.002	0.71	-0.03	-0.13	0.90
Stomach	0.974	0.001	0.74	0.002	0.006	0.99
Intestine	0.971	0.002	0.71	-0.04	-0.19	0.85
Spleen	0.972	0.001	0.72	0.02	0.26	0.80
Visceral organs	1.000	0.0008	1.00	0.40	1.61	0.12
Adipose depots	0.934	0.0006	0.40	-0.15	-1.94	0.07

Table 5.3: Pair-wise phylogenetic least-squares regression (PGLS) between brain and other organ masses, controlling for *whole body mass*, including species from the literature (N=131 species).

	Including *whole body mass* as covariate											
	PGLS						Independent constrasts (λ = 1)			Raw data (λ = 0)		
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	β	t-value	p-value	β	t-value	p-value
Mammals	**N=131**											
Heart	0.943	<0.0001	0.023	0.20	2.16	**0.033**						
Lungs	0.925	<0.0001	0.005	0.15	1.94	0.06						
Kidneys	0.934	<0.0001	0.013	0.10	1.17	0.24						
Liver	0.944	<0.0001	0.021	0.03	0.39	0.70						
Digestive tract	0.945	<0.0001	0.023	0.00	-0.11	0.91						
Visceral tissue	0.946	<0.0001	0.024	-0.02	-0.23	0.82						
Rest	0.946	<0.0001	0.024	0.10	0.21	0.83						
Carnivora	**N=39**											
Heart	0.000	1.00	<0.0001	0.11	0.78	0.44						
Lungs	0.000	1.00	0.0001	-0.02	-0.14	0.89						
Kidneys	0.000	1.00	<0.0001	0.10	0.87	0.39						
Liver	0.000	1.00	<0.0001	0.09	1.03	0.31						
Digestive tract	0.000	1.00	<0.0001	0.00	-0.06	0.95						
Visceral tissue	0.000	1.00	0.0002	0.01	0.08	0.94						
Rest	0.000	1.00	0.0002	-0.18	-0.22	0.83						
Primates	**N=27**											
Heart	0.563	*0.14*	*0.31*	0.34	1.81	0.08	0.21	1.41	0.17	0.49	2.42	**0.023**
Lungs	1.000	0.027	1.00	0.18	1.10	0.28						
Kidneys	0.894	0.015	0.63	0.21	1.39	0.18						
Liver	0.913	0.040	0.80	0.09	0.53	0.60						
Digestive tract	0.688	0.041	0.13	0.23	2.45	**0.022**						
Visceral tissue	0.682	*0.06*	*0.24*	0.36	2.40	**0.025**	0.30	2.07	**0.049**	0.44	2.73	**0.012**
Rest	0.512	*0.13*	*0.06*	-3.58	-2.79	**0.010**	-2.39	-1.87	0.07	-4.33	-3.24	**0.004**
Rodentia	**N=29**											
Heart	0.703	0.001	0.0014	0.26	2.12	**0.044**						
Lungs	0.756	0.0005	0.005	0.02	0.10	0.92						
Kidneys	0.976	0.0008	0.002	-0.06	-0.34	0.73						
Liver	0.760	0.0006	0.002	-0.08	-0.46	0.65						
Digestive tract	0.802	0.002	0.017	0.09	0.61	0.55						
Visceral tissue	0.767	0.0006	0.002	0.05	0.23	0.82						
Rest	0.779	0.0002	0.003	-1.27	-0.85	0.41						

5.2. Pair-wise correlations between organs

Pair-wise correlations between all organs, after controlling for phylogenetic relationships and *fat-free body mass,* for the sample of 100 species are presented in Table 5.4 and Figure 5.1.

We found many significant positive correlations among the visceral organs. Correlations tended to be stronger among thoracic organs and abdominal organs, than between the two types of organs. Brain mass was only positively correlated with stomach mass, and negatively correlated with adipose depot mass (cf. Section 5.1.1). Adipose depot mass was negatively correlated with brain size (cf. Section 5.1.1) and positively correlated with intestine mass, but not with stomach or digestive tract mass.

Figure 5.1: Correlations between the masses of visceral organs, brains and adipose depots in a sample of 100 mammal species, controlling for phylogenetic relationships and fat-free body mass.

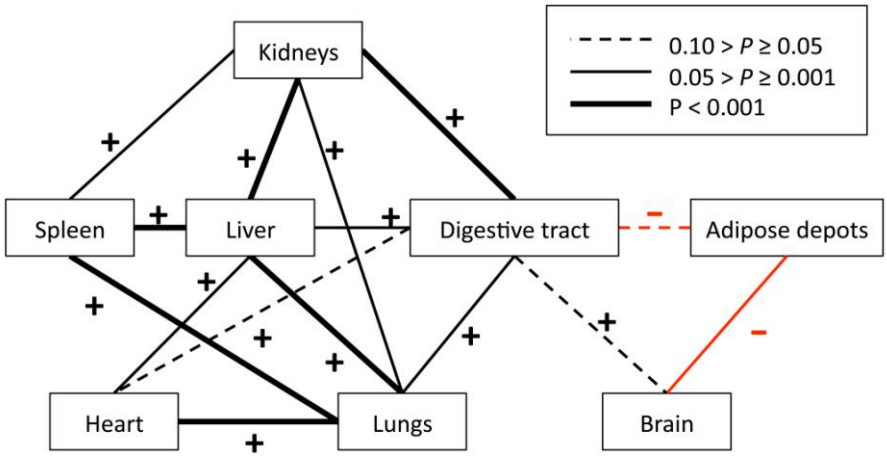


Table 5.4: Pair-wise correlation matrix including brain, visceral organs, and adipose depots mass. All correlations are controlled for *fat-free body mass* and calculated by PGLS (N=100).

	Heart	Lungs	Kidneys	Liver	Digestive tract	Stomach	Intestines	Spleen	Adipose depots
Brain	β = 0.15 P = 0.13	β = -0.03 P = 0.73	β = 0.01 P = 0.92	β = -0.02 P = 0.85	β = 0.16 P = 0.06	β = 0.15 **P = 0.042**	β = 0.11 P = 0.15	β = -0.02 P = 0.60	β = -0.07 **P = 0.017**
Heart		β = 0.63 **P<0.0001**	β = 0.15 P = 0.13	β = 0.18 **P = 0.035**	β = 0.16 P = 0.09	β = 0.08 P = 0.27	β = 0.12 P = 0.13	β = 0.05 P = 0.15	β = -0.03 P = 0.32
Lungs			β = 0.27 **P=0.004**	β = 0.32 **P<0.0001**	β = 0.18 **P = 0.042**	β = 0.06 P = 0.36	β = 0.14 P = 0.07	β = 0.13 **P<0.0001**	β = -0.003 P = 0.93
Kidneys				β = 0.45 **P<0.0001**	β = 0.29 **P = 0.002**	β = 0.06 P = 0.40	β = 0.28 **P=0.0002**	β = 0.11 **P = 0.003**	β = 0.005 P = 0.88
Liver					β = 0.30 **P = 0.005**	β = 0.06 P = 0.43	β = 0.26 **P = 0.004**	β = 0.15 **P<0.0001**	β = -0.01 P = 0.74
Digestive tract						β = 0.53 **P<0.0001**	β = 0.81 **P<0.0001**	β = 0.05 P = 0.19	β = -0.07 P = 0.07
Stomach							β = 0.39 **P<0.0001**	β = 0.04 P = 0.37	β = -0.03 P = 0.55
Intestine								β = 0.04 P = 0.39	β = -0.11 **P = 0.013**
Spleen									β = 0.11 P = 0.24

5.3. Principal Component Analysis (PCA)

Residuals of organ mass (derived from fat-free body mass) clustered together in three factors with eigenvalues larger than 1. Two of these factors grouped the abdominal organs and the thoracic organs, respectively. The third factor loaded negatively on brain size and positively on adipose depots mass (see Table 5.5).

Table 5.5: Principal component analysis (PCA) of organ mass in the 100 species sample, using the residuals of all organs vs. fat-free body mass. Eigenvalues (a) and rotated factor patterns (b). Spleen was not included here.

a)

Number	Eigenvalue	Percent
1	**2.106**	**30.081**
2	**1.366**	**19.518**
3	**1.111**	**15.872**
4	0.909	12.986
5	0.727	10.392
6	0.492	7.027
7	0.289	4.124

b)

Rotated Factor Pattern	Factor 1	Factor 2	Factor 3
Heart (res.)	0.0065	**0.8863**	-0.0815
Lungs (res.)	0.2840	**0.8542**	0.0798
Kidneys (res.)	**0.7456**	0.1800	0.1520
Liver (res.)	**0.7363**	0.2701	0.0048
Digestive tract (res.)	**0.6277**	-0.1443	-0.3071
Adipose depots (res.)	-0.2352	0.1341	**0.7882**
Brain (res.)	-0.3316	0.2614	**-0.6047**

5.4. Effects of sex, provenience and habitat on the correlation between brain size and adipose depots mass

5.4.1. Habitat effects

Controlling for either *whole body mass* (see Table 5.6) or *fat-free body mass* (see Table 5.7), no significant correlations were found between brain mass and the mass of other organs in the temperate subsample, with the exception of a positive correlation between brain mass and stomach mass in the N=45 species sample, after controlling for *whole body mass*. In the tropical subsample, we found positive correlations between brain size and heart, lungs, kidneys and intestines. Some of these correlations persisted even if we controlled for *fat-free body mass* (heart and intestines, see Table 5.7). No negative correlation between brain mass and adipose depot mass was found in the tropical sample, probably due to the fact that half of the species of the tropical subsample were primates.

5.4.2. Provenience effects

When controlling for *whole body mass*, the significant correlations found were positive: between brain size and heart mass in captives, and between brain size and stomach mass in wild individuals (see Table 5.8). A significant negative correlation between brain size and adipose depot mass was found in both subsamples, although the correlation was stronger in wild animals.

When controlling for *fat-free body mass* (see Table 5.9), no positive correlations between brain mass and other organ masses were found, and only the significantly negative correlation between brain mass and adipose depot mass in wild specimens persisted.

Table 5.6: Pair-wise phylogenetic least-squares regression (PGLS) between brain and other organ masses, controlling for *whole body mass*, for a) species with temperate distribution, and b) species with tropical distribution. The left panel corresponds to the sample of 100 species, the right panel corresponds to the sample of 45 species.

a) Temperate	N=52						N=39					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Heart	0.940	<0.0001	0.043	0.23	1.57	0.12	0.961	<0.0001	0.17	0.26	1.47	0.15
Lungs	0.920	<0.0001	0.026	0.06	0.38	0.70	0.938	<0.0001	0.05	0.04	0.21	0.83
Kidneys	0.924	<0.0001	0.027	0.03	0.25	0.80	0.936	<0.0001	0.050	0.09	0.48	0.63
Liver	0.923	<0.0001	0.023	0.06	0.51	0.61	0.937	<0.0001	0.035	0.10	0.71	0.48
Digestive tract	0.930	<0.0001	0.037	0.11	0.89	0.38	0.952	<0.0001	0.13	0.15	0.87	0.39
Stomach	0.925	<0.0001	0.041	0.23	1.92	0.06	0.980	<0.0001	0.58	0.36	2.09	**0.044**
Intestine	0.928	<0.0001	0.032	0.04	0.38	0.71	0.945	<0.0001	0.07	0.08	0.51	0.61
Spleen	0.940	<0.0001	0.08	-0.03	-0.60	0.55	0.950	<0.0001	0.09	-0.03	-0.64	0.52
Visceral organs	0.926	<0.0001	0.024	0.13	0.76	0.45	0.942	<0.0001	0.045	0.17	0.78	0.44
Adipose depots	0.919	<0.0001	0.09	-0.14	-3.17	**0.003**	0.942	<0.0001	0.21	-0.15	-3.10	**0.004**

b) Tropical	N=43						N=4					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Heart	0.939	<0.0001	0.28	0.28	2.43	**0.020**						
Lungs	0.966	<0.0001	0.83	0.30	2.04	**0.048**						
Kidneys	0.915	<0.0001	0.05	0.28	2.03	**0.049**						
Liver	0.921	<0.0001	0.10	0.02	0.14	0.89						
Digestive tract	1.000	<0.0001	1.00	0.34	3.55	**0.001**						
Stomach	0.966	<0.0001	0.80	0.14	1.57	0.12						
Intestine	1.000	<0.0001	1.00	0.31	3.56	**0.0009**						
Spleen	0.942	<0.0001	0.08	0.07	1.21	0.23						
Visceral organs	0.986	<0.0001	0.73	0.33	2.32	**0.026**						
Adipose depots	0.933	<0.0001	0.21	-0.06	-1.33	0.19						

Table 5.7: Pair-wise phylogenetic least-squares regression (PGLS) between brain and other organ masses, controlling *for fat-free body mass*, for a) species with temperate distribution, and b) species with tropical distribution. The left panel corresponds to the sample of 100 species, the right panel corresponds to thesample of 45 species.

a) Temperate	N=52						N=39					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Heart	0.946	<0.0001	0.09	0.14	0.97	0.34	0.954	<0.0001	0.13	0.12	0.69	0.50
Lungs	0.943	<0.0001	0.13	-0.07	-0.47	0.64	0.950	<0.0001	0.15	-0.09	-0.53	0.60
Kidneys	0.940	<0.0001	0.07	-0.09	-0.65	0.52	0.946	<0.0001	0.07	-0.03	-0.19	0.85
Liver	0.937	<0.0001	0.07	-0.01	-0.12	0.91	0.945	<0.0001	0.07	0.02	0.19	0.85
Digestive tract	0.938	<0.0001	0.07	0.02	0.20	0.85	0.947	<0.0001	0.10	0.02	0.12	0.90
Stomach	0.937	<0.0001	0.09	0.16	1.40	0.17	0.967	<0.0001	0.35	0.20	1.16	0.25
Intestine	0.938	<0.0001	0.06	-0.04	-0.34	0.74	0.944	<0.0001	0.08	-0.02	-0.12	0.91
Spleen	0.955	<0.0001	0.18	-0.03	-0.81	0.42	0.957	<0.0001	0.14	-0.03	-0.76	0.45
Visceral organs	0.936	<0.0001	0.07	-0.02	-0.13	0.90	0.945	<0.0001	0.07	-0.01	-0.07	-0.95
Adipose depots	0.933	<0.0001	0.12	-0.08	-2.07	**0.043**	0.951	<0.0001	0.24	-0.10	-2.37	**0.023**

b) Tropical	N=43						N=4					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Heart	0.945	<0.0001	0.36	0.25	2.03	**0.049**						
Lungs	0.970	<0.0001	1.00	0.26	1.73	0.09						
Kidneys	0.922	<0.0001	0.08	0.26	1.98	0.05						
Liver	0.932	<0.0001	0.18	0.00	-0.01	0.99						
Digestive tract	1.000	<0.0001	1.00	0.31	2.17	**0.003**						
Stomach	0.979	<0.0001	1.00	0.14	1.59	0.12						
Intestine	1.000	<0.0001	1.00	0.28	3.09	**0.004**						
Spleen	0.946	<0.0001	0.15	0.05	0.91	0.37						
Visceral organs	0.995	<0.0001	0.89	0.31	2.12	**0.041**						
Adipose depots	0.934	<0.0001	0.23	-0.01	-0.24	0.810						

Table 5.8: Pair-wise phylogenetic least-squares regression (PGLS) between brain and other organ masses, controlling for *whole body mass*, for a) individuals kept in captivity, and b) wild individuals. The left panel corresponds to the sample of 100 species, the right panel corresponds to the sample of 45 species.

a) Captive	N=59						N=8					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Heart	0.791	<0.0001	0.003	0.25	2.12	**0.039**	0.000	1.00	0.018	0.28	1.27	0.26
Lungs	0.766	<0.0001	0.002	0.13	1.04	0.30	0.000	1.00	0.010	0.30	1.22	0.28
Kidneys	0.777	<0.0001	0.001	0.19	1.50	0.14	0.000	1.00	0.012	-0.38	-0.96	0.38
Liver	0.784	<0.0001	0.002	0.07	0.69	0.50	0.000	1.00	0.029	0.08	0.31	0.77
Digestive tract	0.826	<0.0001	0.011	0.23	1.98	0.05	0.000	*1.00*	*0.08*	-0.38	-0.71	0.51
Stomach	0.816	<0.0001	0.010	0.12	1.38	0.17	0.000	1.00	0.008	-0.30	-1.24	0.27
Intestine	0.823	<0.0001	0.009	0.19	1.96	0.06	0.000	*1.00*	*0.11*	-0.10	-0.22	0.83
Spleen	0.794	<0.0001	0.004	0.01	0.12	0.90	0.000	1.00	0.018	-0.08	0.24	0.77
Visceral organs	0.780	<0.0001	0.002	0.22	1.45	0.15	0.000	1.00	0.019	0.38	0.54	0.61
Adipose depots	0.825	<0.0001	0.021	-0.09	-2.10	**0.041**	0.000	1.00	0.018	-0.03	-0.33	0.75

b) Wild	N=39						N=35					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Heart	0.954	<0.0001	0.13	0.28	1.45	0.16	0.949	<0.0001	0.11	0.22	1.00	0.32
Lungs	0.934	<0.0001	0.031	0.01	0.07	0.95	0.934	<0.0001	0.040	0.01	0.04	0.97
Kidneys	0.933	<0.0001	0.031	0.10	0.53	0.60	0.931	<0.0001	0.035	0.10	0.46	0.65
Liver	0.934	<0.0001	0.026	0.03	0.19	0.85	0.934	<0.0001	0.031	0.03	0.20	0.85
Digestive tract	0.958	<0.0001	0.16	0.24	1.58	0.12	0.966	<0.0001	0.27	0.28	1.59	0.12
Stomach	0.965	<0.0001	0.30	0.34	2.39	**0.022**	0.976	<0.0001	0.53	0.42	2.31	**0.027**
Intestine	0.948	<0.0001	0.08	0.13	0.91	0.37	0.955	<0.0001	0.13	0.18	1.15	0.26
Spleen	0.940	<0.0001	0.06	-0.01	-0.25	0.80	0.948	<0.0001	0.10	-0.03	-0.60	0.55
Visceral organs	0.940	<0.0001	0.033	0.24	1.06	0.30	0.940	<0.0001	0.039	0.25	0.94	0.36
Adipose depots	0.959	<0.0001	0.35	-0.16	-3.37	**0.002**	0.942	<0.0001	0.26	-0.18	-3.37	**0.002**

Table 5.9: Pair-wise phylogenetic least-squares regression (PGLS) between brain and other organ masses, controlling for *fat-free body mass*, for a) individuals kept in captivity, and b) wild individuals. The left panel corresponds to the sample of 100 species; the right panel corresponds to the sample of 45 species.

a) Captive	N=59						N=8					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Heart	0.809	<0.0001	0.009	0.20	1.65	0.10	0.000	1.00	0.015	0.28	1.36	0.23
Lungs	0.799	<0.0001	0.009	0.06	0.42	0.67	0.000	1.00	0.008	0.30	1.37	0.23
Kidneys	0.792	<0.0001	0.004	0.14	1.11	0.27	0.000	1.00	0.013	-0.41	-1.06	0.34
Liver	0.808	<0.0001	0.009	0.02	0.21	0.84	0.000	1.00	0.032	0.06	0.25	0.81
Digestive tract	0.837	<0.0001	0.020	0.18	1.56	0.12	0.000	*1.00*	*0.14*	-0.44	-0.85	0.43
Stomach	0.829	<0.0001	0.020	0.11	1.27	0.21	0.000	1.00	0.018	-0.17	-0.73	0.50
Intestine	0.832	<0.0001	0.018	0.14	1.42	0.16	0.000	*1.00*	*0.26*	-0.25	-0.53	0.62
Spleen	0.811	<0.0001	0.011	0.00	0.04	0.97	0.000	1.00	0.024	0.03	0.12	0.91
Visceral organs	0.802	<0.0001	0.006	0.13	0.86	0.40	0.000	1.00	0.019	0.34	0.57	0.59
Adipose depots	0.823	<0.0001	0.019	-0.03	-0.77	0.44	0.000	1.00	0.019	0.020	0.26	0.81

b) Wild	N=39						N=35					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Heart	0.952	<0.0001	0.11	0.17	0.88	0.39	0.945	<0.0001	0.09	0.09	0.42	0.68
Lungs	0.945	<0.0001	0.09	-0.08	-0.48	0.63	0.945	<0.0001	0.12	-0.11	-0.57	0.57
Kidneys	0.942	<0.0001	0.05	0.00	0.02	0.99	0.941	<0.0001	0.06	-0.01	-0.05	0.96
Liver	0.942	<0.0001	0.06	-0.02	-0.13	0.90	0.940	<0.0001	0.07	-0.02	-0.14	0.89
Digestive tract	0.958	<0.0001	0.16	0.17	1.10	0.28	0.960	<0.0001	0.22	0.18	1.00	0.33
Stomach	0.963	<0.0001	0.26	0.27	1.90	0.07	0.968	<0.0001	0.39	0.30	1.60	0.12
Intestine	0.949	<0.0001	0.09	0.07	0.50	0.62	0.953	<0.0001	0.14	0.11	0.68	0.50
Spleen	0.953	<0.0001	0.13	-0.02	-0.52	0.60	0.961	<0.0001	0.22	-0.05	-0.94	0.36
Visceral organs	0.943	<0.0001	0.05	0.10	0.43	0.67	0.942	<0.0001	0.06	0.07	0.25	0.81
Adipose depots	0.964	<0.0001	0.39	-0.120	-2.74	**0.010**	0.951	<0.0001	0.31	-0.14	-2.78	**0.009**

5.4.3. Sex effects

If the samples were split by sex and controlled for *whole body mas*s, positive correlations between brain size and the mass of the heart (in females) and the digestive tract (in males and females) were found (see Table 5.10). In both samples, we only found a negative correlation between brain mass and adipose depot mass.

When controlling for *fat-free body mass* (see Table 5.11), the only remaining significant positive correlation was between heart mass and brain size in females in the sample of 100 species. Here, males did not show any correlation between brain size and adipose depot mass, whereas in females and wild females the correlation remained significantly negative. In the sample of 45 species, brain size and adipose depot mass were negatively correlated in both males and females. Overall, the negative correlation between brain size and adipose depot mass was stronger in females in comparison to males, and stronger in wild females in comparison to all females.

Table 5.10: Pair-wise phylogenetic least-squares regression (PGLS) between brain and other organ masses, controlling for *whole body mass*, for a) males, b) females, and c) wild females. The left panel corresponds to the sample of 100 species, the right panel corresponds to the sample of 45 species.

a) Male	N=69						N=33					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Heart	0.866	<0.0001	0.0005	0.12	1.04	0.30	0.826	0.001	0.002	0.13	0.68	0.50
Lungs	0.866	<0.0001	0.0005	0.05	0.37	0.71	0.940	0.001	0.003	0.22	1.23	0.23
Kidneys	0.864	<0.0001	0.0005	0.06	0.52	0.60	0.821	0.0009	0.002	0.12	0.61	0.55
Liver	0.865	<0.0001	0.0005	-0.02	-0.22	0.83	0.823	0.001	0.002	0.08	0.46	0.65
Digestive tract	0.912	<0.0001	0.010	0.18	2.04	**0.046**	0.911	0.0002	0.12	0.28	2.04	0.05
Stomach	0.883	<0.0001	0.001	0.13	1.47	0.15	0.867	0.0005	0.019	0.21	1.21	0.23
Intestine	0.912	<0.0001	0.011	0.14	1.91	0.06	0.905	0.0002	0.10	0.23	1.93	0.06
Spleen	0.866	<0.0001	0.0006	0.00	0.05	0.96	0.826	0.001	0.003	-0.02	-0.38	0.71
Visceral organs	0.880	<0.0001	0.001	0.12	0.89	0.38	0.865	0.0004	0.014	0.32	1.35	0.19
Adipose depots	0.901	<0.0001	0.004	-0.11	-1.75	**0.008**	0.867	0.0002	0.004	-0.16	-2.79	**0.009**

b) Female	N=57						N=30					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Heart	0.892	<0.0001	0.001	0.54	3.50	**0.0009**	0.995	<0.0001	0.89	0.64	3.18	**0.004**
Lungs	0.860	<0.0001	0.003	0.22	1.46	0.15	0.946	0.0005	0.12	0.11	0.50	0.62
Kidneys	0.897	<0.0001	0.003	0.30	1.99	0.05	0.956	<0.0001	0.15	0.45	2.20	**0.036**
Liver	0.998	0.0001	0.009	0.07	0.59	0.56	0.951	0.0007	0.10	0.10	0.65	0.52
Digestive tract	0.954	<0.0001	0.07	0.36	2.94	**0.005**	0.989	<0.0001	0.64	0.37	2.32	**0.028**
Stomach	0.944	<0.0001	0.027	0.34	2.75	**0.008**	0.979	<0.0001	0.55	0.40	1.95	0.06
Intestine	0.949	<0.0001	0.07	0.26	2.44	**0.018**	0.983	<0.0001	0.45	0.29	2.08	**0.047**
Spleen	0.891	0.0001	0.016	0.01	0.15	0.89	0.955	0.0005	0.16	0.00	-0.03	0.97
Visceral organs	0.896	<0.0001	0.003	0.35	1.97	0.05	0.960	0.0001	0.12	0.38	1.71	0.10
Adipose depots	0.920	<0.0001	0.002	-0.15	-3.78	**0.0004**	1.000	0.0001	1.00	-0.16	-5.22	**<0.0001**

c) Wild females	N=28						N=25					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Heart	0.985	<0.0001	0.64	0.58	2.72	**0.011**	0.987	0.0001	0.72	0.61	2.41	**0.025**
Lungs	0.944	0.0004	0.14	-0.04	-0.18	0.86	0.956	0.001	0.29	-0.11	-0.41	0.69
Kidneys	0.946	0.0001	0.11	0.33	1.44	0.16	0.946	0.0002	0.12	0.44	1.61	0.12
Liver	0.940	0.0008	0.07	0.00	0.02	0.98	0.946	0.002	0.11	0.00	0.02	0.98
Digestive tract	0.963	0.0002	0.25	0.26	1.35	0.19	0.981	0.0003	0.57	0.34	1.58	0.13
Stomach	0.960	<0.0001	0.27	0.33	1.71	0.10	0.966	0.0004	0.41	0.35	1.36	0.19
Intestine	0.954	0.0003	0.14	0.15	0.85	0.41	0.974	0.0004	0.37	0.25	1.32	0.20
Spleen	0.928	0.0004	0.07	0.04	0.47	0.64	0.939	0.001	0.12	0.03	0.26	0.80
Visceral organs	0.944	0.0003	0.06	0.28	1.00	0.33	0.950	0.0007	0.09	0.29	0.93	0.36
Adipose depots	1.000	0.001	1.00	-0.15	-4.18	**0.0003**	1.000	0.001	1.00	-0.16	-4.42	**0.0002**

Table 5.11: Pair-wise phylogenetic least-squares regression (PGLS) between brain and other organ masses, controlling for *fat-free body mass*, for a) males, b) females, and c) wild females. The left panel corresponds to the sample of 100 species, the right panel corresponds to the sample of 45 species.

a) Males	N=69						N=33					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Heart	0.883	<0.0001	0.001	0.07	0.65	0.52	0.842	0.001	0.003	0.08	0.40	0.69
Lungs	0.885	<0.0001	0.002	-0.02	-0.12	0.90	0.849	0.0007	0.003	0.17	0.91	0.37
Kidneys	0.884	<0.0001	0.002	0.00	0.03	0.98	0.841	0.0005	0.004	0.05	0.25	0.80
Liver	0.884	<0.0001	0.002	-0.05	0.54	0.59	0.842	0.001	0.003	0.06	0.38	0.71
Digestive tract	0.915	<0.0001	0.013	0.14	1.46	0.15	0.909	0.0002	0.10	0.23	1.62	0.12
Stomach	0.894	<0.0001	0.003	0.10	1.08	0.28	0.871	0.0004	0.015	0.16	0.93	0.36
Intestine	0.915	<0.0001	0.013	0.11	1.38	0.17	0.904	0.0002	0.08	0.19	1.52	0.14
Spleen	0.884	<0.0001	0.002	0.01	0.17	0.87	0.845	0.0005	0.004	-0.01	-0.19	0.85
Visceral organs	0.889	<0.0001	0.002	0.05	0.34	0.73	0.868	0.000	0.011	0.23	1.01	0.32
Adipose depots	0.905	<0.0001	0.006	-0.06	-1.68	0.10	0.876	0.0002	0.006	-0.12	-2.13	**0.041**

b) Females	N=57						N=30					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Heart	0.905	<0.0001	0.003	0.40	2.57	**0.013**	0.986	<0.0001	0.61	0.42	1.88	0.07
Lungs	0.908	<0.0001	0.026	0.06	0.40	0.69	0.975	0.0002	0.55	-0.16	-0.81	0.43
Kidneys	0.913	<0.0001	0.007	0.18	1.27	0.21	0.962	0.0001	0.19	0.25	1.14	0.27
Liver	0.922	<0.0001	0.025	-0.02	-0.18	0.86	0.963	0.001	0.22	-0.01	-0.07	0.94
Digestive tract	0.953	<0.0001	0.06	0.25	1.97	0.05	0.981	0.0002	0.48	0.20	1.18	0.25
Stomach	0.945	<0.0001	0.026	0.24	1.98	0.05	0.970	0.0002	0.37	0.16	0.74	0.47
Intestine	0.947	<0.0001	0.05	0.17	1.48	0.14	0.980	0.0002	0.40	0.16	1.12	0.27
Spleen	0.930	<0.0001	0.025	-0.03	-0.41	0.68	0.970	0.0002	0.32	-0.04	-0.53	0.60
Visceral organs	0.914	<0.0001	0.009	0.15	0.82	0.42	0.964	0.001	0.18	0.12	0.52	0.61
Adipose depots	0.934	<0.0001	0.006	-0.090	-2.45	**0.017**	1.000	<0.0001	1.00	-0.12	-4.27	**0.0002**

c) Wild females	N=28						N=25					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Heart	0.978	<0.0001	0.49	0.43	1.93	0.06	0.980	0.0002	0.54	0.42	1.57	0.13
Lungs	0.964	0.0004	0.45	-0.20	-0.93	0.36	0.992	0.001	1.00	-0.32	-1.50	0.15
Kidneys	0.949	0.0004	0.13	0.17	0.73	0.47	0.952	0.0008	0.16	0.25	0.87	0.39
Liver	0.945	0.002	0.14	0.06	-0.39	0.70	0.954	0.005	0.21	-0.06	-0.36	0.73
Digestive tract	0.957	0.0007	0.22	0.13	0.67	0.51	0.971	0.001	0.42	0.17	0.74	0.46
Stomach	0.956	0.0003	0.23	0.21	1.10	0.28	0.960	0.0009	0.31	0.15	0.56	0.58
Intestine	0.949	0.0009	0.15	0.04	0.23	0.82	0.968	0.001	0.33	0.13	0.65	0.52
Spleen	0.943	0.0004	0.17	0.01	0.06	0.95	0.956	0.0008	0.28	-0.01	-0.15	0.89
Visceral organs	0.946	0.003	0.11	0.06	0.21	0.84	0.953	0.006	0.16	0.04	0.14	0.89
Adipose depots	1.000	0.0004	1.00	-0.120	-3.42	**0.002**	1.000	0.0004	1.00	-0.13	-3.68	**0.0013**

5.5. Correlations between brain size and organ masses in the sample with one species per subfamily

In this sample, the significantly negative correlation between brain size and adipose depots mass was confirmed, although the p-value was slightly higher than in the N=100 species dataset (see Table 5.12). Otherwise, no significant correlations between brain size and the mass of any visceral organs were found.

Table 5.12: Pair-wise phylogenetic least-squares regression (PGLS) between brain and other organ masses (N=51 species, one per subfamily), controlling for fat-free body mass.

Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Heart	0.936	<0.0001	0.46	0.08	0.59	0.56
Lungs	0.929	<0.0001	0.42	0.02	0.13	0.90
Kidneys	0.959	<0.0001	0.56	0.25	1.69	0.10
Liver	0.943	<0.0001	0.51	0.07	0.56	0.58
Digestive tract	0.929	<0.0001	0.42	0.01	0.06	0.95
Stomach	0.933	<0.0001	0.45	-0.05	-0.42	0.68
Intestine	0.941	<0.0001	0.51	0.05	0.44	0.66
Spleen	0.920	<0.0001	0.38	-0.03	-0.42	0.68
Visceral organs	0.941	<0.0001	0.51	0.08	0.42	0.68
Adipose depots	0.904	<0.0001	0.24	-0.10	-2.06	**0.045**

5.6. Correlations with basal metabolic rate (BMR)

5.6.1. Brain size vs. basal metabolic rate

Our results showed a strong positive correlation between brain size and basal metabolic rate (BMR), independent of the variable used to control for body size (see Table 5.13). When we tested possible correlations between BMR and other organs, only kidney mass correlated positively after controlling for *whole body mass*. On the other hand, a significant negative correlation between adipose tissue mass and BMR was found. This correlation persisted when we controlled for *fat-free body mass*.

5.6.2. Including basal metabolic rate (BMR) as a covariate in the brain vs. organ correlations

When BMR was included as covariate in the models, it had a significant effect on brain size, but the mass of the visceral organ included in the model suddenly did not correlate with brain size (see Table 5.14 a) and b)). Adipose depot mass was significantly negatively correlated with brain size, but only if we controlled for *whole body mass*. Moreover, if adipose depots mass was included in the model, BMR did not have a significant effect on brain size.

Table 5.13: Pair-wise phylogenetic least-squares regression (PGLS) between BMR and organ masses (N=64 species), controlling for a) *whole body mass*, and b) *fat-free body mass*.

	a) Including *whole body mass* as covariate					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Brain	0.957	<0.0001	0.41	0.30	2.77	**0.007**
Heart	0.479	0.0090	0.007	0.11	1.07	0.29
Lungs	0.433	0.015	0.001	0.04	0.40	0.69
Kidneys	0.272	0.15	<0.0001	0.19	2.08	**0.042**
Liver	0.000	1.00	<0.0001	0.04	0.39	0.70
Digestive tract	0.500	0.12	0.015	0.19	1.76	0.08
Stomach	0.923	0.0001	0.79	0.17	1.27	0.21
Intestine	0.000	1.00	0.002	0.18	1.55	0.13
Spleen	0.814	0.003	0.005	0.29	1.10	0.27
Visceral organs	0.000	1.00	0.0001	0.15	1.89	0.06
Adipose depots	0.181	0.35	<0.0001	-0.77	-3.07	**0.003**

	b) Including *fat-free body mass* as covariate					
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value
Brain	0.960	<0.0001	0.41	0.24	2.28	**0.026**
Heart	0.619	0.0008	0.024	0.02	0.21	0.83
Lungs	0.531	0.002	0.001	-0.06	-0.60	0.55
Kidneys	0.276	0.14	<0.0001	0.12	1.29	0.20
Liver	0.000	1.00	<0.0001	-0.02	-0.20	0.84
Digestive tract	0.542	0.19	0.020	0.12	1.11	0.27
Stomach	0.951	0.0002	0.82	0.09	0.70	0.49
Intestine	0.000	1.00	0.001	0.12	1.06	0.29
Spleen	0.806	0.004	0.004	0.23	0.88	0.38
Visceral organs	0.277	0.47	0.0001	0.04	0.47	0.64
Adipose depots	0.150	0.49	<0.0001	-0.81	-3.02	**0.004**

Table 5.14: Pair-wise phylogenetic least-squares regression (PGLS) between brain and organ masses (N=64 species), controlling for BMR, for a) *whole body mass*, and b) *fat-free body mass*.

	a) Including *whole body mass* as covariate						
Organ	λ	P (λ=0)	P (λ=1)	Variable	β	t-value	p-value
Heart	0.970	<0.0001	0.51	Organ	0.23	1.85	0.07
				BMR	0.26	2.41	**0.019**
Lungs	0.955	<0.0001	0.37	Organ	0.08	0.60	0.55
				BMR	0.29	2.66	**0.010**
Kidneys	0.955	<0.0001	0.36	Organ	0.12	0.96	0.34
				BMR	0.27	2.38	**0.021**
Liver	0.959	<0.0001	0.41	Organ	0.05	0.51	0.61
				BMR	0.29	2.73	**0.008**
Digestive tract	0.973	<0.0001	0.54	Organ	0.16	1.35	0.18
				BMR	0.28	2.57	**0.013**
Stomach	0.974	<0.0001	0.59	Organ	0.13	1.23	0.22
				BMR	0.29	2.61	**0.011**
Intestine	0.971	<0.0001	0.51	Organ	0.13	1.28	0.21
				BMR	0.28	2.62	**0.011**
Spleen	0.960	<0.0001	0.49	Organ	-0.03	-0.58	0.56
				BMR	0.30	2.81	**0.007**
Visceral organs	0.965	<0.0001	0.44	Organ	0.15	1.06	0.29
				BMR	0.28	2.59	**0.012**
Adipose depots	0.937	<0.0001	0.23	Organ	-0.11	-2.55	**0.013**
				BMR	0.19	1.67	0.10

Table 5.14 continued:

	b) Including *fat-free body mass* as covariate						
Organ	λ	P (λ=0)	P (λ=1)	Variable	β	t-value	p-value
Heart	0.967	<0.0001	0.46	Organ	0.16	1.22	0.23
				BMR	0.23	2.18	**0.034**
Lungs	0.960	<0.0001	0.42	Organ	-0.01	-0.04	0.97
				BMR	0.23	2.26	**0.027**
Kidneys	0.959	<0.0001	0.38	Organ	0.07	0.58	0.57
				BMR	0.22	2.11	**0.039**
Liver	0.961	<0.0001	0.42	Organ	0.01	0.14	0.89
				BMR	0.24	2.27	**0.027**
Digestive tract	0.970	<0.0001	0.50	Organ	0.10	0.82	0.41
				BMR	0.23	2.22	**0.030**
Stomach	0.971	<0.0001	0.54	Organ	0.09	0.85	0.40
				BMR	0.23	2.24	**0.030**
Intestine	0.969	<0.0001	0.48	Organ	0.08	0.81	0.42
				BMR	0.23	2.24	**0.029**
Spleen	0.963	<0.0001	0.49	Organ	-0.03	-0.58	0.56
				BMR	0.24	2.31	**0.024**
Visceral organs	0.965	<0.0001	0.44	Organ	0.07	0.49	0.62
				BMR	0.23	2.25	**0.028**
Adipose depots	0.948	<0.0001	0.29	Organ	-0.06	-1.62	0.11
				BMR	0.17	1.49	0.14

5.7 Summary

Our results provided no evidence for a physiological trade-off between brain mass and other expensive tissue mass. When controlling for *whole body mass*, the correlation between brain mass and some organs was positive (significantly or not), but these positive correlations mostly disappeared when we controlled for *fat-free body mass*.

On the other hand, we found a negative correlation between brain mass and adipose depot mass in mammals. This correlation was robust when we used different models to control for body size effects. Within orders, the negative correlation was most expressed in rodents, but completely absent in primates.

When species with approximated adipose depot masses were excluded from the analyses, correlations between brain size and the size of other organs were still not found, whereas the negative correlation between brain mass and adipose depot mass persisted. The negative correlation between brain size and adipose depots mass was more significant in females, in wild-caught specimens, and in species of temperate origin, and was most pronounced in the subset of wild females.

When basal metabolic rate (BMR) was included in the analyses as a proxy of total metabolic turnover, it correlated positively with brain size and negatively with adipose depot mass. The only variable which still showed an effect on brain size when BMR was included in the model as a covariate was adipose depot mass.

Chapter 6: Discussion

In the present study, we tested the Expensive Tissue and the Energy Trade-off Hypothesis using reliable morphological data and controlling for both phylogeny and metabolism.

6.1. Old datasets

First, we conducted a re-evaluation of the original analysis that was used to provide support for the Expensive Tissue Hypothesis in anthropoid primates (Aiello and Wheeler 1995). In contrast to the original result of Aiello and Wheeler, our revised sample did not yield any significantly negative correlations between brain and the digestive tract variables (stomach and intestines) or the combined digestive tract mass (see Section 2.3). Results did not differ according to whether or not phylogenetic information was taken into account and whether or not the two strepsirrhine species were included in the sample.

There may be various reasons for the discrepancy between these results and the originally reported negative correlation. First, the Harvey dataset reported some brain size values that were not confirmed in subsequent reports (Isler *et al*. 2008), and sometimes reported only male values without mentioning this fact. Second, sexual size dimorphism affects body mass more than brain mass (Plavcan 2001), which may confound analyses where sex is not taken into account. Third, brain size data have become available for more platyrrhine species in recent years, reducing the bias toward catarrhine species in the original analysis. Fourth, we excluded clearly emaciated individuals of the Chivers dataset in our analysis. In conclusion, matching the best available brain and body mass data with the Chivers dataset did not yield support for the Expensive Tissue Hypothesis in anthropoid primates. However, the absence of a negative correlation was not

sufficient to reject the Expensive Tissue Hypothesis because the unmatched data could simply suffer from a high degree of error variation.

From the later evaluation of the published datasets, it was evident that the Expensive Tissue Hypothesis, both in mammals and in primates, could be only reliably tested using newly collected morphological measures.

6.2. Our sample

Our experimental setup to control for preservation effects showed that treatment after death influences the mass of the different organs in laboratory mice (see Chapter 3). Cold treatment did not influence body mass, but led to a slight decreased or increase in the mass of several visceral organs. Alcohol treatment, on the other hand, caused a pronounced decrease of tissue mass. The calculated correction factors did not prove to be sufficiently accurate in a test with another rodent species of similar body size. Therefore, we did not include alcohol-preserved specimens in our analyses. Our sample contained mainly frozen specimens.

After two years of data collection, we built a dataset with complete measurements of 100 mammalian species, including 23 primate species. We complemented this dataset with morphological measurements from the literature, obtaining a combined dataset of 131 species, and with BMR data from the literature for 64 of these species. With these data, we tested the relationship between body composition, metabolism and brain size in mammals.

6.3. The Expensive Tissue Hypothesis refuted

The original Expensive Tissue Hypothesis (Aiello and Wheeler 1995) predicted a physiological trade-off between brain mass and the masses of other expensive tissues, especially

the mass of the digestive tract. Contrary to the predictions of the Expensive Tissue Hypothesis, we found no negative correlations between the relative size of the brain and the digestive tract, other expensive organs or their combined sum among mammals or within non-human primates, controlling for fat-free body mass, even though the statistical power of our analyses was sufficient to detect these negative correlations if they existed (see Section 4.1). We also did not find any trade-offs amongst other expensive organs (see Section 5.1). Moreover, controlling for whole body mass many correlations between brain size and organ masses were indeed positive, showing that the manner of controlling for body size is relevant in such analyses.

These results therefore refute the Expensive Tissue Hypothesis as a general principle to explain the interspecific variation of relative brain size in mammals. In my view, this finding reduces the plausibility of the argument that human encephalization was made possible by a reduction of the digestive tract (Aiello and Wheeler 1995; Aiello *et al*. 2001).

Energy trade-offs with other tissues that are less expensive but very abundant (Isler and van Schaik 2006), may nonetheless have explained part of brain size variation. One of these tissues, skeletal muscle mass, was considered by Aiello and Wheeler (1995), but was excluded by the argument that a high amount of muscle mass would have had to be sacrificed to explain the enlargement of brain mass in *Homo*. Although we were not able to test the correlation between brain mass and skeletal muscle mass in our sample, we could at least test a possible correlation between brain mass and other cheap and abundant tissue in mammals, the adipose tissue. Our results (see Section 5.1) show a negative correlation between brain mass and adipose depot mass. This correlation is corroborated by a principal component analysis (see Section 5.3), which shows that brain mass clusters negatively with adipose depot mass.

We assumed that sex, habitat and provenience (wild vs. captive) could have an influence on the correlation between brain size and other tissues (see Section 4.3). No change was observed in the correlations between brain mass and the masses of other visceral organs, when we compared males vs. females, captive vs. wild specimens and tropical vs. temperate species. The negative correlation between brain mass and adipose depot mass, however, showed some variation between different subsamples. Females showed a more significant negative correlation than males. Wild animals showed a more significant negative correlation than captive animals. The negative correlation was strongest in the wild female subsample. These results suggest that reducing error variation and controlling for potentially confounding variables strengthens the evidence for a brain size-adipose depot trade-off.

Within tropical species brain size and adipose depot mass were not correlated. This result may either indicate that the trade-off between these tissues is not found in more stable environments, or it may be a consequence of the problematic data for primates (see below), which make up a large proportion of the tropic subsample. Future work must show whether the trade-off between brain and adipose depots is also found among tropical mammals.

The strongest trade-off between fat storage and brain size evolution is expected in taxa that exhibit high cost of transport for increased whole body mass, such as climbing or flying mammals and birds. The only animals that can easily combine both strategies of fat storage and brain enlargement may be those that do not face increased cost of transport for increased whole body mass, e.g. aquatic mammals or large bipeds (Garland 1983). However, more detailed studies of seasonal variation in body mass are needed to investigate which conditions or lifestyles favour one or the other, or a combination of both strategies.

Adipose depots make up an appreciable proportion of body mass in some mammals (Pond 1998). Fat stores enable animals to cope with periods of reduced food intake and thus act as a physiological buffer against starvation. On the other hand, relatively large brains have also been proposed to act as cognitive buffers against starvation (Sol 2009; van Woerden *et al*. 2010). It is therefore possible that encephalization and fat storage are complementary strategies to buffer against starvation. Most species either accumulate fat or develop big brains. We think that this is caused by the fact both strategies are costly. Big brains need more energy, and adipose tissue, although not metabolically expensive, has an energetic cost because it has to be carried around and may increase predation-induced mortality.

6.4. Costs of locomotion

Interspecifically, the metabolic cost of transport in animals increases with body mass, as demonstrated by Taylor and Heglund (1982) for a wide range of animals. The cost of transport per gram body mass decreases with body mass$^{-0.33}$, and thus the cost of transport of the whole body increases with body mass$^{0.66}$. However, the relationship varies between orders or taxa groups, and there seems to be a steeper increase in rodents (N=15 species) and primates (N=10, including *Tupaia* and *Homo sapiens*) than in artiodactyls (N=10), carnivores (N=11) or marsupials (N=11; Taylor and Heglund 1982). In any case, this interspecific relationship is likely to yield an underestimate of the metabolic cost of transport of additional adipose depots, as the musculoskeletal system does not grow in concert with additional body mass from fat storage.

Overall, the energy spent on locomotion varies widely between individuals of a species, depending on season, reproductive state, food availability and other variables. It can be estimated from day ranges and time spent on locomotion (cf. Garland 1983; Leonard and Robertson 1997),

although day range is usually underestimating actual path length, which has a fractal dimension, and time spent on locomotion is confounded by the fact that some locomotion is also needed for other activities such as foraging or social life. Small animals of less than 500 g body mass spend only about 1% of their total energy on locomotion, whereas this value increases to 5-15% in larger animals (Garland 1983).

These values may seem to be rather small. However, the actual cost of carrying adipose depots may not only consist of an increase in the direct costs of locomotion, but the addition of an indirect cost of being less swift to escape attacking predators or conspecifics. This cost depends on the lifestyle of the species and may be higher in terrestrial and/or small species. Jumping distance is impaired in fatter cats (Harris and Steudel 2002), and in small monkeys forced to carry additional weights on their trunk (Garber *et al*. 2005). Maximum running speed does not generally increase with body mass within taxa (Garland 1983), and it even decreases in artiodactyls. In human athletes, the fastest and most enduring sportsmen are usually those with the lowest percentage of body fat, if training levels are also taken into account (Knechtle *et al*. 2011). Therefore, it seems justified to assume that an increased percentage of adipose depots, without a parallel increase in the size of the musculoskeletal system, also significantly decreases maximum running speeds in animals.

Extant human foragers spend between 22% and 18% of their daily energy expenditure on locomotion (estimates of Leonard and Robertson 1997 and of Isler and van Schaik 2006, following a model of Pontzer and Wrangham 2004), and chimpanzees between 16% and 30% (same sources). Using the same equations, 10% additional fat stores would increase the percentage of energy used for locomotion about 1% in humans, but 2-3% in chimpanzees (the exact chimpanzee value differs according to whether we use the Taylor (1973) equation for

treadmill running of a juvenile chimpanzee (body mass: 17kg), or the Taylor (1982) equation derived from 10 primate species).

Recently, Hanna and colleagues (2008) have shown that vertical climbing efficiency increases only very slightly with body mass in primates (exponent of 0.11, not significantly different from zero), i.e. the cost of travel during climbing is almost directly proportional to body mass. We therefore expect that animals that include a fair amount of vertical travel are affected more strongly than predominantly terrestrial species by an increase in the size of adipose depots.

In conclusion, direct and indirect costs of adipose depots through their effect on locomotor efficiency are clearly evident, although they probably vary quite considerably between lineages or even more closely-related species. Furthermore, available data and models confirm that the costs of quadrupedal locomotion and vertical climbing in nonhuman apes show a steeper increase with body mass than human walking or running. Efficient bipedalism similar to that of modern humans probably evolved in the first members of the genus *Homo* about 2 million years ago (Pontzer *et al*. 2009). Therefore, we conclude that storing fat would be less costly for an efficient biped such as early *Homo* than for their ancestors, thus reducing a possible trade-off between brain size and the size of adipose depots in this lineage.

6.5. The special case of the primates

The results within primates do not quite fit into the general mammalian picture. Our hypothesis predicted that the negative correlation between brain size and adipose depots mass should also be present in primates as a group, but it was not (cf. Section 5.1). Even if the whole primate order would be following a cognitive buffering strategy in comparison to other mammalian taxa, we still expect that the relationship would be valid within the group, as not all

primate species equally rely on cognitive buffering (van Woerden *et al*. 2010). There are four reasons leading us to conclude that our data of primates does not accurately reflect the trade-off between adipose depots and brain size.

First, as our primate specimens were captives from a variety of husbandry conditions, there may be a large variation in fat storage in our sample which does not reflect "true" biological variation. Seasonality should be less of a concern for captive primates, but age and cause of death may strongly affect adipose depots in unpredictable directions. Indeed, many closely related species in our sample differ considerably in their amount of adipose depots. As tip contrasts have a large impact on the results of phylogenetic methods, they may mask an underlying trend (c.f. Martin *et al*. 2005).

Second, Pond (1998) reported that subcutaneous fat stores are more conspicuous in primates than in other groups, and we know from qualitative comparisons that different primate species store adipose depots at different places in their body. The small-brained fat-tailed dwarf lemurs (*Cheirogaleus medius* and *C. major*) store fat in their tails, increasing their body mass up to 78% before hibernation (Muller 1999). Orangutan males exhibit fatty cheek pads, and probably also store fat around the neck, in addition to abdominal fat stores (Winkler 1989). Even within humans, males differ in fat store distribution from females (Wells 2009). Therefore, our measurements and subsequent scaling of abdominal fat stores may not be accurately estimating total body fat in some primate species, and the resulting error may mask any underlying correlation.

Third, peculiarities of the gastrointestinal tract of foregut fermenters may exert an influence on the capacity to store fat in captive animals. In the order of primates, only the

subfamily Colobinae belongs to this group. In contrast to hindgut fermenters (i.e. all other primates), a diet of energy-dense, low-fiber foods does not increase body mass in this group, but rather leads to a loss of body mass, diarrhea, and premature death (Nijboer 2006). This is a well-known phenomenon in captive colobines, which are extremely sensitive to husbandry conditions and are rarely kept in zoos. Our sample contains two colobine species (*Colobus polykomos* and *Trachypithecus vetulus*). If these are excluded from the analysis, a very weak negative trend between brain size and adipose depots mass appears in the reduced sample (N=21, lambda =0.692, p-value of adipose depots on brain size controlling for body mass: 0.368, beta =-0.072).

Fourth, some of the primate specimens in our sample were rather light-weight in comparison to the wild adult body mass of the respective sex (<75%, *Cebus apella, Macaca nigra, Symphalangus syndactylus, Theropithecus gelada*, and *Mandrillus sphinx*). If we exclude these specimens from the analysis (in addition to the two colobines, see point 3), the negative trend between adipose depots and brain size, controlling for body mass, actually becomes significant (N=16, lambda =0.018, p =0.022, beta =-0.180). The same result is obtained by excluding only one single data point, *Cebus apella* (N=20, lambda =0, p-value of adipose depots on brain size controlling for body mass: 0.022, beta = -0.181). The latter species is represented by a single female individual of only 70% of the normal female body mass of this species. However, its adipose depots mass is, contrary to the overall low body mass, very high in comparison to other primates (51.4 g abdominal fat mass, 1750 g body mass). This leads to an extremely large contrast with its closest relative in our sample, a male *Saimiri boliviensis*, which exhibits a very low value of adipose depots mass (4.85g abdominal fat mass, 1003 g body mass). But how can we know which of the two individuals is an outlier? For *Saimiri*, we have dissected two other specimens of a closely related species, *Saimiri sciureus*, which were not included in the final

sample because their skull was damaged. All three *Saimiri* individuals exhibit a similarly low amount of abdominal adipose depots relative to body mass (less than 1% abdominal fat in all three specimens, as compared to 3% in the *Cebus apella*). It is therefore likely that the *Cebus apella* data point is an outlier, but until more data on *Cebus* are obtained we cannot say whether this is also found in other individuals of the species or genus or whether it is an abnormality of this specimen.

In conclusion, all four points taken together confirm that there is good reason to maintain that the negative correlation between adipose depots and brain size would also be found in primates, if more accurate data could be obtained. Obviously, we need more accurate data to settle this issue.

6.6. The new Expensive Brain framework

Where does refuting the Expensive Tissue Hypothesis leave us with respect to explaining the evolution of the much enlarged human brain? Although there are various cognitive benefits to increased brain size (Reader *et al*. 2011), empirical evidence shows that a focus on the energy costs of growing and maintaining brain tissue helps to explain the interspecific variation in brain size (Martin 1981). This approach had been synthesized in the Expensive Brain Hypothesis (Isler and van Schaik 2009), which incorporates earlier ideas on energetic aspects of brain size evolution (see Section 1.4; Martin 1981; Aiello and Wheeler 1995; Aiello *et al*. 2001). The results from the present study motivated some changes in this framework (see Figure 6.1). Larger brains are sometimes paid for by a permanent increase in net energy intake of an organism, as indexed by its basal metabolic rate (BMR), as shown by the positive correlation between BMR and brain size in a large sample of placental (Isler and van Schaik 2006) and marsupial mammals

(Isler 2011). This was confirmed in the present data set, where we could control for fat-free body mass. We humans exhibit the BMR expected for a mammal or primate of our body mass, but because we have much larger adipose depots (about 14-26% in healthy adults [Wells 2009]) than chimpanzees and bonobos (about 3-10% [Zihlman 1984]), human BMR relative to fat-free body mass is appreciably higher than theirs (Aiello and Wells 2002). Therefore, if extant apes are representative of the last common ancestor, brain enlargement during human evolution was partially paid for through a permanent increase in net energy intake.

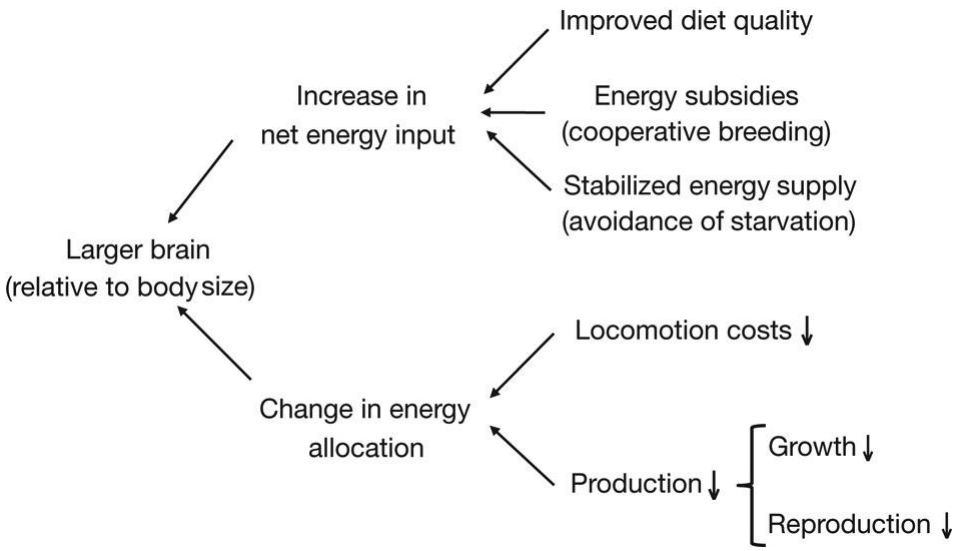


Figure 6.1. The revised Expensive Brain framework.

Starting with Early Pleistocene *Homo*, an increase in relative brain size could have been achieved through different pathways First, they improved diet quality as indicated by increased consumption of meat and bone marrow (Aiello and Wheeler 1995) and by tool-assisted food processing, at one point including cooking (Wrangham 2009). Second, despite having moved into highly seasonal habitats (Potts 1998) they reduced temporal fluctuations in energy budgets by cognitive buffering (Kaplan *et al*. 2000), which is also known for other primates (van Woerden *et*

al. 2010) and birds (Sol 2009). Third, provisioning and food sharing probably arose with the adoption of cooperative breeding and confrontational scavenging among the earlier representatives of the genus *Homo* (Burkart *et al*. 2009; Wrangham 2009). Comparative research suggests that such energy subsidies for reproducing females and dependent offspring can support increased brain size (Isler and van Schaik 2009; Isler 2011).

The second pathway to brain enlargement is increased energy allocation to the brain by savings on other expensive functions, although the Expensive Tissue Hypothesis for organs is no longer supported. One likely trade-off could be found between brain size and the costs of locomotion. The efficient form of bipedal locomotion that arose with the transition from australopithecines to early *Homo* (Pontzer *et al*. 2010) could have led to major reductions in energy expenditure in two ways. On one hand, its low costs in comparison with the climbing and quadrupedal locomotion of nonhuman apes (Pontzer *et al*. 2009) should have lowered daily energy expenditure on locomotion (Isler and van Schaik 2006), and on the other hand, bipedalism may reduce the effect of increased weight due to adipose depots on the energy costs of locomotion. A second potential trade-off would be the one between brain size and production, comprising both growth and reproductive effort, which has been demonstrated for mammals (Isler and van Schaik 2009; Isler and van Schaik 2009). Beginning with early *Homo*, our lineage has increased brain size and reduced the pace of life history (Dean *et al*. 2001).

In sum, I do not claim unique processes operating exclusively in human evolution. All these processes are known to operate among mammals in general. I propose that during human evolution, improved diet quality, allomaternal subsidies, cognitive buffering, reduced locomotion

costs and reduced allocation to production all operated simultaneously, thus enabling the extraordinary brain enlargement in our lineage.

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